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Review

Plant secondary metabolites and its dynamical systems of induction in response to environmental factors: A review

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Under natural conditions, the plants are, usually, surrounded by a series of potential enemies. They had created strategies of defense against pathogens and herbivores' attacks, allowing its perpetuation throughout evolution. These defense mechanisms are closely associated with the synthesis of secondary metabolites, which are also worldwide used in several areas of industry. This bibliographic review was conducted aiming to better understand how plants synthesize these substances in response to biotic or abiotic stimuli. The results discussed here revealed that synthesis of plant secondary metabolites is dependent on changes in several metabolic pathways, being often directly associated with the primary metabolism. Injury by herbivores or pathogens, temperature, CO_2 levels, solar radiation and drought, are the factors related to the environment that express the most significant signs of inhibition or synthesis of plant secondary metabolites. Global climatic changes recently observed can affect the metabolic pathways network and, consequently, secondary metabolites synthesis. Nowadays, genomic tools have been useful alternatives that are leading to a new revolution of plant breeding, allowing the overexpression or inhibition of these substances. Some limitations and challenges to be achieved upon the dynamics of secondary metabolite synthesis in plants are presented.

Key words: Jasmonic acid, transcription factors, inhibition, synthesis.

INTRODUCTION

Plants produce a range of organic compounds which, a priori, have no direct relation to its growth and development. Originating of primary metabolism, these compounds are known as secondary metabolites. These metabolites show no functions hitherto recognized in vital processes of plants, such as photosynthesis, cellular respiration, protein synthesis, solute transport and nutrient assimilation, unlike the primary metabolites. The synthesis of a specific secondary metabolite is not observed in all species of the plant kingdom, being restricted to one or a few species (Taiz and Zeiger, 2010).

For a long time, the importance of secondary metabolites was not fully understood. Nowadays, with advances in research, technology, and especially with the genome sequencing of some species such barley (Mayer et al., 2012), tomato (Consortium, 2012), *Pinus taeda* (Kovach et al., 2010), pear (Wu et al., 2013) and rice (Project, 2005), and with the recently gene-editing technique (Gaj et al., 2013; Woo et al., 2015; Bortesi and Fischer, 2015) many doubts about the role of these compounds in the evolution of the main grown species were clarified.

It is known that biotic factors such as pathogen and herbivores attack, and abiotic such as radiation, temperature, hydric and nutritional stress, have the potential to induce physiological changes in plants by altering metabolic pathways. In this context, the aim of this bibliographic review was to discuss the biosynthetic pathways responsible for the synthesis of the main secondary metabolites, its main functions in plants, and how the environment can influence, positively or negatively, the synthesis of these compounds.

SECONDARY METABOLITES

We now know that much of the secondary metabolites has important functions but not vital in plants, such as its protection to parasites (fungi, insects, bacteria), attractive features (color, odor, taste) for pollinators and seed dispersers, as well as in acting as subjects in plant-plant competition and plant-microorganism symbioses. However, the same metabolites responsible for increasing plant production performance can also make them undesirable for human consumption. As an example, we can consider the production of capsaicin, a capsaicinoid responsible for the pungency in peppers (*Capsicum* spp.). Its synthesis is controlled by a dominant allele locus *Pun1*. In its homozygous recessive state, *pun1 / pun1*, capsaicin's synthesis does not occur (Blum et al., 2002). Different degrees of poignancy observed among genotypes occur so, both due to the environmental effects where the genotype is subjected, as due genotype x environment interaction, being found variations in pungency level among and within genotypes (Zewdie and Bosland, 2000).

This can be explained due to high outcrossing rates (7 to 90%), which may occur among cultivars, although this species be considered autogamous (Bozokalfa et al., 2009). It is known that the genotype x environment interaction is characterized by the differential response of each genotype in each environment; and that this interaction is caused mainly by the variation of meteorological elements of each site. Studies have shown that temperatures above 30°C (Ohta, 1960) and water restriction (Gurung et al., 2011; Sung et al., 2005), increment the capsaicin level in *Capsicum* spp.

Secondary metabolites can be divided into three chemically distinct main groups: terpenes, phenolic compounds and nitrogen compounds.

Terpenes

Terpenes, also known as isoprenoids are the largest class of known secondary metabolites, containing about 50,000 identified substances (Vranová et al., 2012). These substances are formed by the fusion of five carbon units which have a branched backbone. These can be divided into monoterpenes (10 carbons), sesquiterpenes (15 carbons), and diterpenes (20 carbons). The largest terpenes include triterpenes, tetraterpenes and poly-terpenoids, with 30, 40 and > 40 carbons, respectively (Taiz and Zeiger, 2010). Terpenes are synthesized from primary metabolites by at least two different pathways: the mevalonate pathway (joining three molecules of Acetyl-CoA) and the non-mevalonate pathway, which both produce Isopentenyl diphosphate (IPP) and Dimethylallyl diphosphate (DMAPP), respectively, being the basic units in the synthesis of terpenes (Figure 1). As can be noted, the basic source of terpenes and other secondary metabolites is directly related to the primary metabolism of plants. In this case, the presence of Acetyl-CoA at the pathway of IPP and Pyruvate plus Glyceraldehyde 3-Phosphate, at the pathway of DMAPP, is essential for the synthesis of terpenoids in plants.

Several plants show a mix of terpenes with volatile compounds, which confer to they a specific odor. Among some species, it can be highlighted the lemon, mint, basil and sage. These compounds, known as essential oils, can be extracted from plants, being commercially important in pharmaceutical, cosmetic and food industries.

Some terpenes show important functions on growth and development of plants, such as gibberellins (diterpenes) and brassinosteroids (triterpenes). Though some terpenes show metabolic importance, a great part of them, are basically associated with the defense of several plants against herbivores as insects (Trapp and Croteau, 2001; Veitch et al., 2008) and nematodes (Soriano et al., 2004).

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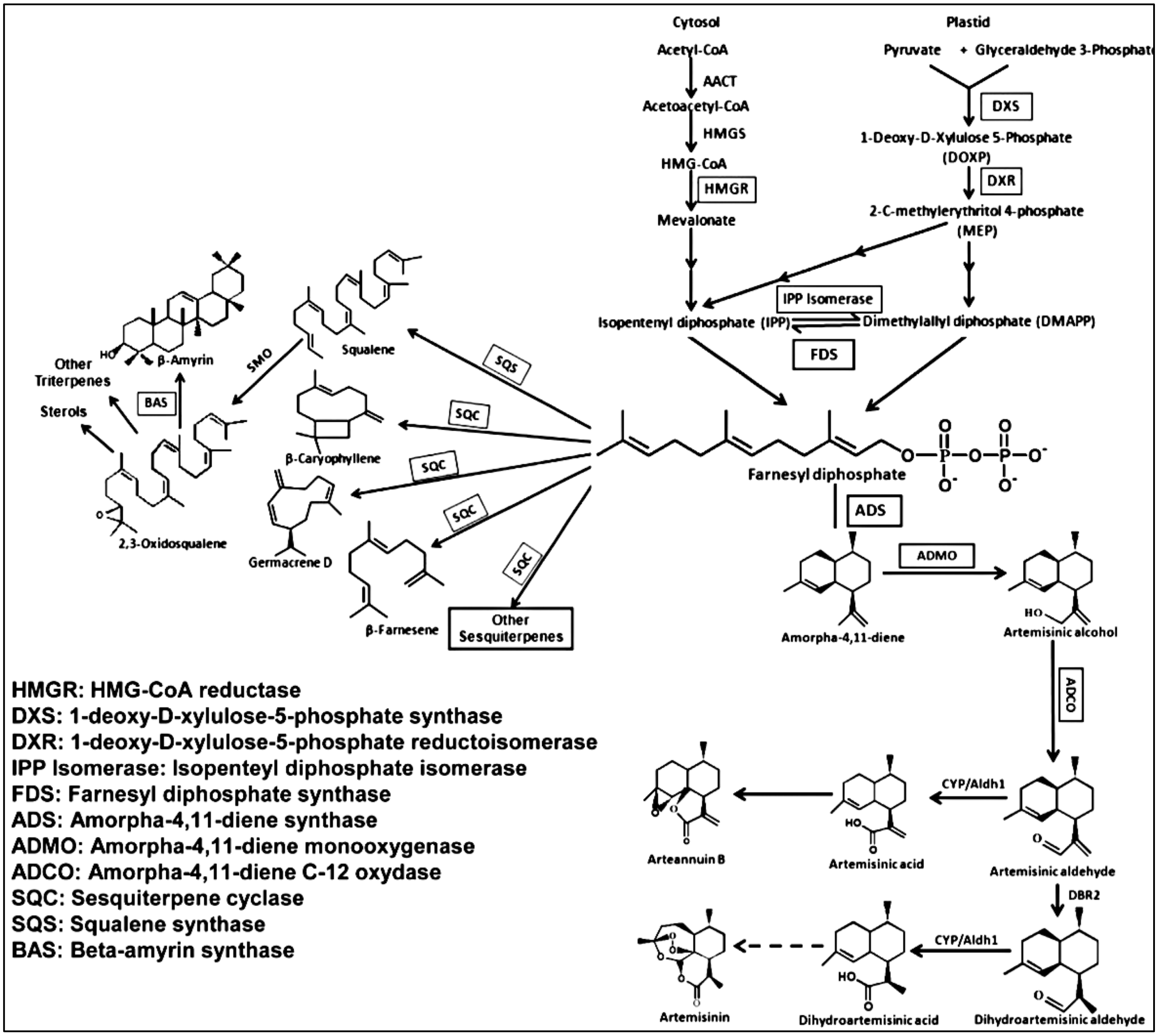

Figure 1. Schematic representation of the biosynthetic pathway of some terpenoids. Observe the two basic pathways at the beginning of the synthesis: the IPP pathway held into cytosol of the cell, and the DMAPP pathway, held in the plastids. Source: Adapted from Yadav et al. (2014).

In some conifers, resins exudation, secondary metabolite belonging to the group of terpenes, is presented as a defense mechanism against fungi and insects (Franceschi et al., 2005), and is used as a raw material for a wide range of industrial products, being one of the most important non-wood forestry products (Hall et al., 2013). Its synthesis can be induced by environmental factors such as high temperatures, radiation and evapotranspiration (Rodríguez-García et al., 2015), by exogenous application of chemical stimulants such as jasmonates (Dar et al., 2015; Moreira et al., 2009, 2012) or by mechanical damage (Ruel et al., 1998). Research has shown that weather conditions can affect resin production of two ways: directly, by changing fluidity of the resin due to higher temperature (Blanche et al., 1992) and indirectly, due of changes in the physiological processes involved in the biosynthesis and secretion of this substance (Genoa et al., 2013).

Effects of some environmental factors on the concentration of terpenes in tomato were revealed, indicating that high temperatures (above 32°C) significantly reduces the amounts of some tetraterpenoids as lycopene, if this stress is induced during late stages of fruit maturation. High temperatures since the vegetative stage, however, show no changes in the composition of these substances in relation to the control treatment, indicating metabolism's acclimation of these plants to high temperatures (Hernández et al., 2015). The influence of controlled water stress, may be related to an increase of lycopene in tomatoes, as evidenced by (Wang et al., 2015a), which reveals the dynamic and complex synthesis of these compounds, dependent on environmental factors.

Several transcription factors of secondary metabolite biosynthesis, suggests that expression of activators or repressors in response to phytohormones and environmental signage is responsible for the synthesis and accumulation of such compounds. Recent research has revealed some of the metabolic pathways are also controlled by posttranscriptional and posttranslational mechanisms (Patra et al., 2013). Recent studies in *Artemisia annua*, a species of Artemisia genus, originating from temperate regions of Asia, revealed that several transcription factors activated by jasmonate phytohormone, leading to artemisinin synthesis, a sesquiterpene widely used in the treatment of malaria (Yu et al., 2012). Studies have shown that drought negatively modulates the production of artemisinin in this plant mainly by reducing the density and size of glandular trichomes, however, the synthesis of some monoterpenes and volatile compounds is induced by water deficit (Yadav et al., 2014).

Jasmonic acid (JA) presents itself as an important signaling molecule of secondary metabolites synthesis, triggering the emission of volatile products such as homoterpenes, like the emission of volatile compounds observed after injury caused by herbivores (Arimura et al., 2000; Hopke et al., 1994; Nabity et al., 2013). Research has revealed that JA is responsible for the increase in secondary metabolites levels in more than thirty species, mainly due to signaling of genes responsible for the synthesis of enzymes such as phenylalanine ammonia-lyase, known to be involved in the chemical plant defense mechanism (Dar et al., 2015; Gundlach et al., 1992). Exogenous applications of JA can act as a tolerance inducer of some plants to salt stress (Qiu et al., 2014), where even in situations of stress of an isolated plant, for example, increasing in concentration of this substance can induce the expression of genes defense in the surrounding plants, even these being from different families (Farmer and Ryan, 1990).

The increase in endogenous concentration of JA is mainly determined by response of plants to environmental stimuli such as high luminosity (Li et al., 2014), low CO_2 levels (Sun et al., 2013.), water stress (Zhang and Huang, 2013) and high levels of toxic substances (Yan et al., 2015). The complexity of the interactions between environmental factors and responses of plants has been stimulating conducting research aimed at obtaining superior genotypes tolerant to adverse climatic conditions, mainly due to prospects of global warming and increases in CO_2 levels, expected for not too distant future.

Phenolic compounds

Plants produce a large variety of secondary metabolites which contain a hydroxyl functional group into an aromatic ring; such substances are known as phenolic compounds. Phenols are a large group of compounds which can be divided into five subgroups: coumarins, lignins, flavonoids, phenolic acids and tannins. Among these, lignins and flavonoids are the most pervasive phenolic compounds in plants (Gumul et al., 2007).

In superior plants, the biosynthesis of phenolic compounds is associated with two basic metabolic pathways: the shikimic acid pathway and the malonic acid pathway. Most of the phenolic compounds are derived from phenylalanine, a product of the shikimic acid pathway, which is converted into cinnamic acid by phenylalanine ammonia-lyase (PAL), perhaps the most studied enzyme in plant secondary metabolism.

In many species, PAL activity regulation becomes complex due the existence of multiple genes that encode this enzyme, some of which are only expressed in specific tissues or under certain environmental conditions, revealing the complex dynamics of signaling and synthesis of secondary metabolites (Cheng et al., 2015; De Jong et al., 2015; Logemann et al., 1995).

Coumarins

Coumarins are benzene and dipirona fused rings with great therapeutic importance. Depending on your configuration has a great ability in regulating cellular routes that can be exploited for cancer prevention (Thakur et al., 2015), besides having great influence on the central nervous system (Skalicka-Woźniak et al., 2015). Despite advances in research with this phenolic compound are not found specific results regarding the influence of environment factors in its synthesis or inhibition.

Lignins

Lignins are very important in plants' sustaining, due to its ability to provide greater rigidity to the cell wall. Although several synthetic polymers are used in a range of applications, the dependence of crude oil for its manufacture has resulted in several environmental impacts, encouraging scientists on finding alternative raw materials for this purpose (Obaid et al., 2016). Lignins, due to its specific structure, it is becoming an environmentally friendly substance with large utilities in the industry as the manufacture of molds, thermoplastic reinforcement and integration with natural fibers to obtain the most varied materials (Saheb et al., 1999).

The synthesis of this substance in plants seems to be related to the presence of sucrose, causing significant intracellular disorder, irregular thickening of the cell wall and lignification. The response to lignification, however, is changed due to the activity of H_2O_2, being lignin synthesis temporarily correlated with the synthesis of this peroxide (Nose et al., 1995). Many are the works evaluating the behavior of different types of lignin, particularly for the thermal behavior, degradation products and processing methods of these substances, but still needs more information that reveals how the interaction between the species and growth environment influences the synthesis of these substances.

Flavonoids

Flavonoids are the major class of plant phenolic compounds. The basic skeleton of these substances contains 15 carbons arranged in two aromatic rings connected by a three-carbon bridge. Besides to promoting pigmentation in flowers, flavonoids are important in protecting plants against UV rays, besides to attracting pollinators and seed dispersers. In addition to the beneficial effect on plants, some research has shown its importance in humans' diet, acting as a potent antioxidant (Winkel, 2004). Flavonoids can be classified into four subgroups, due to the degree of oxidation of the carbon chain, as follows: anthocyanins, flavones, flavonols and isoflavones:

Anthocyanins

Anthocyanins are one of the most important flavonoids studied at the physiology, mainly acting in plant-pollinator-dispersers interaction, due to its ability to provide color to plants. These compounds are essential for the plants being associated with cold and pathogens (Sivankalyani et al., 2016) and when present in the human diet may act as antioxidant (Homoki et al., 2016; Sui et al., 2016).

By the year 2007, it has been suspected that JA regulates plant metabolism in response to many stressful situations, such as the attack of pathogens or insects, drought, or extreme changes in ambient temperature, triggering a series of gene expression, however, JA transmission mechanism was not known in detail. Research has identified a family of repressor proteins called jasmonate-ZIM-domain proteins (JAZ), that bind to transcription factors of several defense mechanisms and prevents them from acting. When the plant identifies any stress situation (injury, temperature, drought), JA is synthesized as an alarm signal which binds to a receptor and induces degradation of JAZ by ubiquitin-proteasome 26S system, as shown in Figure 2 (Chini et al., 2007; Thines et al., 2007). Genetic engineering techniques have been effective in increasing JA biosynthesis. An example was shown when genes responsible by transcription factors that regulate JA responses were overexpressed, resulting in a greater accumulated amount of terpenoids (Fits and Memelink, 2000).

Low temperatures induce expression of specific genes in the metabolic pathway of synthesis of key enzymes, such as PAL, retarding the degradation of these substances as well as flavonoids and polyphenols (Rouholamin et al., 2015; Zhang et al., 2015). Small RNAs (miRNAs) are important components of a gene-regulatory network, being involved in anthocyanin biosynthesis. Studies using Arabidopsis, has revealed that the expression of genes encoding key enzymes in the biosynthesis of these substances is regulated by specific transcription factors, and its synthesis is induced by JA in the presence of light, but not in dark, being dependent on the phytochrome's response on red-distant light stimuli (LI et al., 2014).

Genetic and physiological evidence has shown that the Myb regulation (Figure 2) by JA triggers an immediate accumulation of anthocyanin and trichomes initiation. Overexpression of MYB75 leads to accumulation of anthocyanin in Arabidopsis *coi1-1* mutants, compared to the wild-type genotype (WT), in the same way that overexpression of GL3 and EGL3 increments the number of trichomes in this plant (Figure 3).

In Arabidopsis, there is an accumulation of anthocyanin mainly at the junction of the rosette and stem. This spatial pattern is controlled by miRNA156, which have a relationship with SPL9, a key regulator of plant development which promotes flowering (Figure 2). When increased activity of miRNA156 occurs, there is an expression of genes that increase the synthesis of anthocyanins; on the other hand, the reduced activity of miRNA156 promotes greater expression of SPL9, resulting in higher levels of flavonoids. This interaction reveals a direct relationship between the transition to flowering stage and secondary metabolism of plants, providing crucial information for handling anthocyanins and flavonoid content in plants (Gou et al., 2011).

Studies with *Potamogeton gramineus* L., an aquatic plant found in irrigation canals in Northern California, has revealed some effects of environmental factors in the synthesis of anthocyanins. Under nitrogen and phosphorus limitation, these substances are inhibited, while the temperature and luminosity slightly increase its content and reduces the chlorophyll content, which seems to be related to the reddish-brown color of this species (Spencer and Ksander, 1990). We saw the complex dynamics of synthesis and inhibition of these compounds in plants and, taking into account the importance of these substances, it is clear the difficulty, particularly regarding the breeders, in finding genotypes with high anthocyanin production and acclimatized in varied environments.

Flavones and flavonols

These substances are found in all organs of plants, with

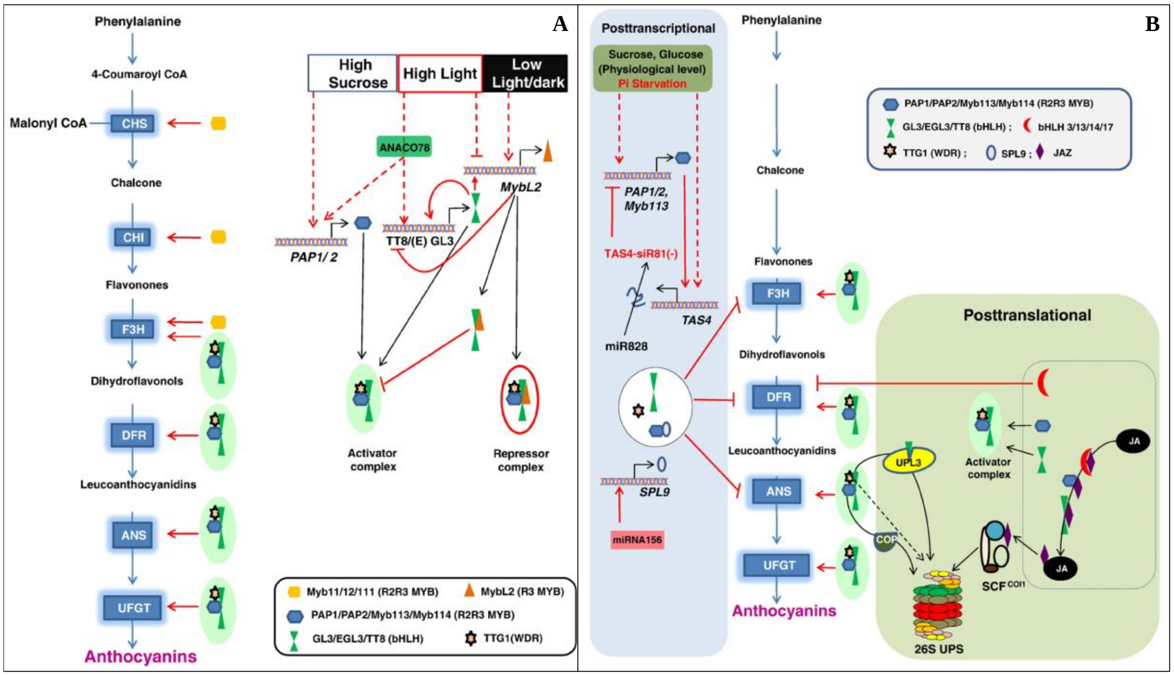

Figure 2. (A) Schematic representation of gene regulation of anthocyanin biosynthesis in response to environmental signals in Arabidopsis. Genes encoding pathway enzymes are represented in blue boxes. solid red arrows and T-bars, represent the direct activation and repression, respectively. Dotted red arrows and T-bars indicate indirect activation and repression, respectively. Half-circle indicates self-activation. The activation of genes by Myb-bHLH-WDR complex (green elliptical circle) and Myb11 / 12/111 (solid yellow rectangle) is shown. The red circle represents the elliptical repressor complex Myb-bHLH-WDR. (B) Schematic representation of posttranscriptional and posttranslational regulation of anthocyanin biosynthesis. The structural genes encoding the enzymes of the route are shown in blue boxes. solid red arrows and T-bars represent the direct repression and activation, respectively. Dotted red arrows and T-bar indicate indirect activation and repression, respectively. The blue shaded area shows the anthocyanin biosynthesis regulated by miRNAs in response to environmental signals and development. The gray shaded area represents a posttranslational degradation of components of the Myb-bHLH-WDR and JAZ proteins by the 26S ubiquitin proteasome system. Source: Adapted from Patra et al. (2013).

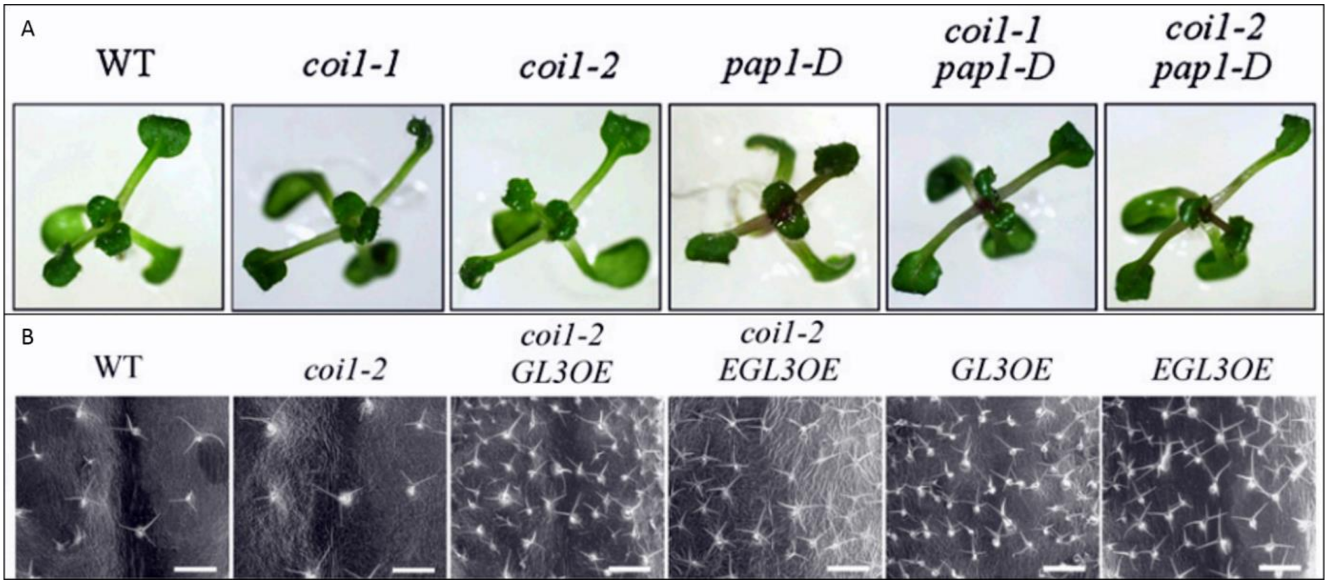

Figure 3. (A) Overexpression of MYB75 in Arabidopsis coi1-1 mutants resulting in increased anthocyanin; (B) Overexpression of GL3 and EGL3, resulting in increased trichome number in coi1-2. Source: Adapted from QiI et al (2011).

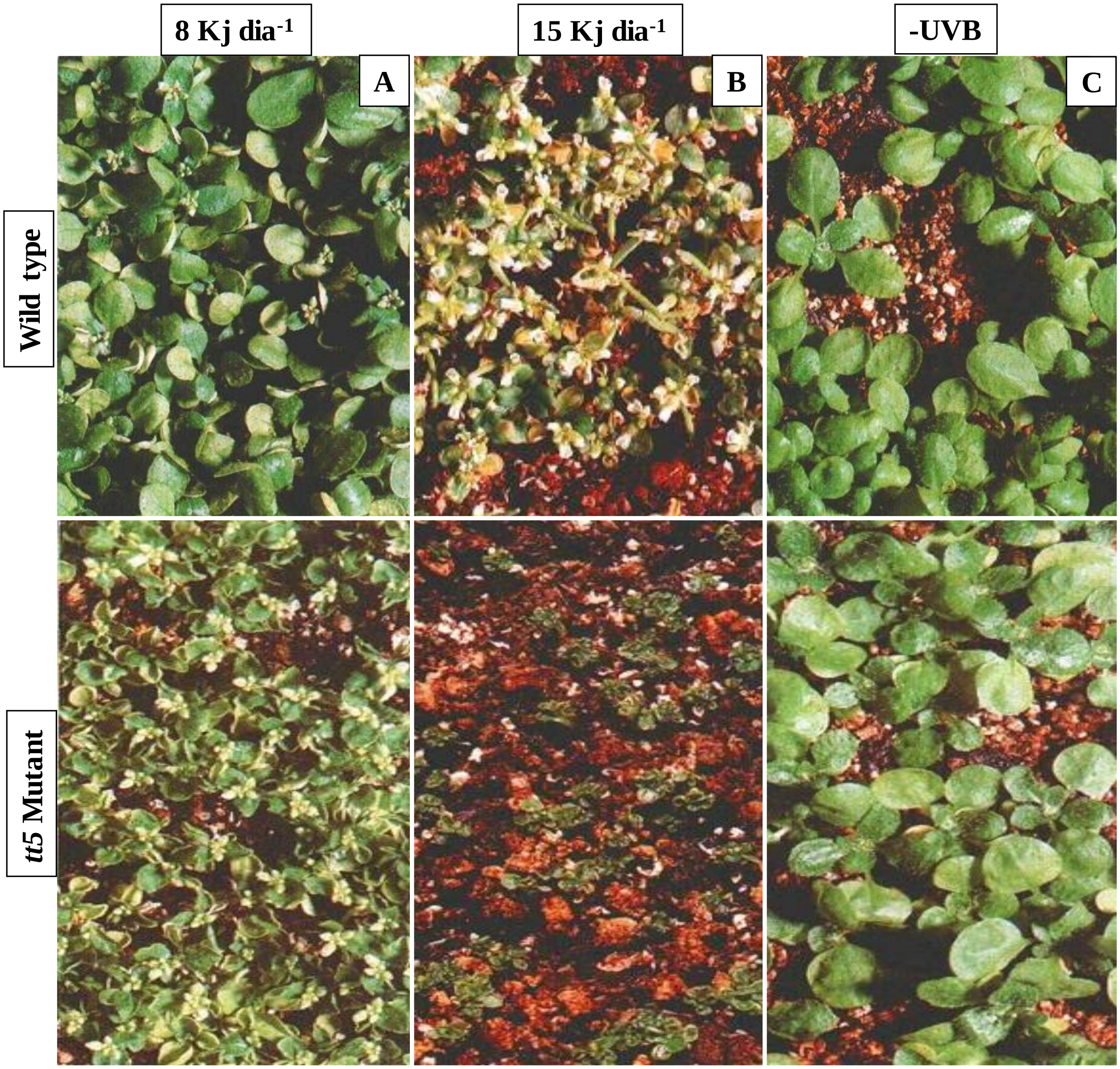

Figure 4. Growth of Arabidopsis *tt5* mutants and wild types under 8 kj day^{-1} (a), 15 kj day^{-1} (B) and on without UVB radiation wavelength (C). Source: Adapted from Li et al. (1993).

special importance for the flowers. Usually, these substances absorb light-long waves that are lower to the visual ability of human beings. However, insects like bees, which are responsive to light in the ultraviolet range, are attracted by these colorations.

Flavones and flavonols have great importance to plants, due to their ability to protect plants against damage from ultra-violet light (280-320 nm) mainly due be presents in the epidermis of leaves and stem, absorbing light at this wavelength (Taiz and Zeiger, 2010).

In Arabidopsis mutant for the synthesis of these compounds, plants are hypersensitive to UVB radiation, grown less under normal condition. When protected, these plants have similar growth to the wild-type genotype. Figure 4 clarifies the role of this sub-group of phenolic compounds. Incident radiation with the presence of UVB, even at low density, causes the standstill of growth in Genotypes TT5 mutants, as compared to the wild-type. When the incident light has no radiation in the UVB wavelength, both the wild type as mutant for the synthesis of flavones and flavonols, have similar growth.

It is visible the influence of radiation in the mutant plants for synthesis of these substances. Thus, a challenge to be achieved is to obtain genotypes that have high concentrations of these protective substances, thus avoiding a reduction in the growth and development due to the inhibitory effect of radiation, especially with the prospect of ozone layer depletion, an important molecule responsible for filtering the vast majority of incident UV radiation.

Isoflavones

These substances are mainly found in Fabaceae species. With 745 genera and over 19,500 species, these legumes are the third largest family of plants. Among several biological activities of these substances, we can mention the beneficial effect on the initial growth of *Trachinotus ovatus*, a species of fish of Carangidae family (Zhou et al., 2015), increase in cell antioxidant activity (Al-Qudah et al., 2015), as well as a reduction of up to 27% in anti-*Tobacco Mosaic Virus* (Li et al., 2015).

Recent research has aroused the interest of isoflavones for their action as phytoalexins. These compounds are generally absent in healthy plants, and its synthesis is induced by plant responses to biotic factors, including infection by fungi and bacteria (Budovská et al., 2013; Ejike et al., 2013), or abiotic factors, such as physical injury (Darvill and Albersheim, 1984). Studies have also shown that there is a possibility of increasing the resistance of plants to diseases based on an additional external application of phytoalexins (Stuiver and Custers, 2001).

One of the first studies evaluating the influence of the environment factors in response to the synthesis of these substances has revealed that when plants are adapted to metalliferous environments, their response to biotic stress (fungi) is more intense, resulting in increased production of phytoalexins (Martellini et al., 2014). This example of positive interaction between biotic and abiotic stimuli can arouse the interest of researchers, particularly regarding the production of plants with higher levels of phytoalexins.

Phenolic acids

These phenolic compounds are characterized by being formed by a benzene ring, a carboxyl group and one or more hydroxyl groups in their molecules, giving them antioxidant properties, both for the foods, as for the bodies, being indicated for treatment and prevention of a number of diseases, among them cancer (Chang et al., 2015; Espinosa et al., 2015; Heleno et al., 2015; Kurth et al., 2015; Milner, 2002; Taofiq et al., 2015).

Salicylic acid

It is known the effect of salicylic acid in some plant functions, such as reducing ethylene levels and increasing capsaicin content of peppers (Sudha and Ravishankar, 2003), as well the increasing anthocyanin production in carrots (Rajendran et al., 1992). However, this substance is basically related signaling of plant resistance to pathogens.

Localized attack by a pathogen induces acquired resistance, mainly due to the accumulation of salicylic acid, which induces the accumulation of proteins related to the elimination of pathogens. Research has proved the efficiency of induced resistance in plants through genetic modification techniques and has demonstrated the ability of a leaf (resistant) promote long-distance signaling and induce resistance in the surrounding plants. This process is basically related to the interaction of ‘resistant’ leaf with a derived-lipid molecule (Maldonado et al., 2002) and with methyl silicate production, a volatile liquid synthesized from salicylic acid (Shulaev et al., 1997).

Few are studies about the influence of environmental factors in synthesis or inhibition of these compounds. Breeding programs have indicated that a large diversity of phenolic acids is found in more than 60 varieties of vegetables and wild relatives. The heritability estimates for these substances have relatively high (> 0.5), indicating that selection for higher levels of phenolic acids can be effective without major environmental influences (Prohens et al., 2007). With the advancement of plant breeding techniques, genetic tools such as transposon handling and gene silencing also have been shown to be effective in increasing the synthesis of phenolic acids in plants, without effects on the levels of other compounds. This suggests the possibility of improvement in antioxidant capacity and plant resistance, without changes in agronomic traits of the species (Kaushik et al., 2015; Niggeweg et al., 2004).

Tannins

Tannins along with lignin are considered one of the most important groups of secondary metabolites in the defense of plants, mainly due to their biochemical and molecular properties (Adamczyk et al., 2013; Zucker, 1983). There are basically two categories, namely condensed tannins, compounds formed by the addition of flavonoid constituents of woody plants and the water-soluble tannins, polymers that present phenolic acids and simple sugars (Taiz and Zeiger, 2010).

Herbivores such as cattle, deer, monkeys and birds avoid plants or parts of them that have higher tannin levels. Studies have shown that interaction of these substances with proteins, for example, determines the selectivity of some herbivores in plant feeding (Clausen et al., 1990; Zungu and Downs, 2015).

The plant defense strategies are intrinsically related to the organ in which the tannins are synthesized. Studies with *Medinilla magnifica*, known as rose grape, revealed the cells that synthesize tannins show distribution patterns defined in plant organs with a preference for accumulation around or near the vascular tissues. In leaves, these substances tend to accumulate in mesophyll region and surrounding tissues, with the highest concentration in the apical region. Roots and stems tend to have lower levels of these substances, yet still found in small quantities.

The concentration of tannins in reproductive structures and fruit maturity was also relatively high, reducing the extent which fruit maturing (Robil and Tolentino, 2015). This specific distribution both spatially and temporally may indicate its role in protecting vital structures of plants and also an obvious adaptation to make more palatable fruit only when they are physiologically mature and presenting viable seeds, leading to success in the spread of species.

In addition to its role in herbivores food selectivity, these compounds may also have antibiotic activity. A condensed tannin isolated from *Gossypium hirsutum* flower, known as Mexican cotton, was presented as an antibiotic component of this plant, comprising about 3.4% of the dry weight of the flower. Its use in the diet of *Heliothis virescens*, tobacco caterpillar, retarded growth of the larvae in 84% (Chan et al., 1978). However, recent studies have revealed that addition of specific isolates of these substances in flour for cooking, improves their properties, primarily due to increased hydrogen bonding, improving the interactions between proteins and carbohydrates, resulting in a more compact gluten (Wang et al., 2015b).

There are few studies that reveal how the environment provides changes in synthesis of these compounds, however, some research has shown that tannins can significantly influence the soil changes. The addition of these substances decreases carbon mineralization and nitrogen nitrification, significantly affecting the complexation of nitrogen compounds and consequently the availability of nitrogen (Adamczyk et al., 2013).

Nitrogen compounds

A great variety of secondary metabolites has nitrogen in their structures. The main nitrogenous compounds found in plants are alkaloids, glycosides, and non-protein amino acids: these are usually synthesized from the common amino acids (Taiz and Zeiger, 2010). In this session will be addressed the main types of nitrogenous compounds produced by the plants, their biological properties and how environmental factors can influence their synthesis.

Alkaloids

Alkaloids are a large group with more than 15,000 substances, being found in about 20% of the vascular species. These compounds are generally characterized by the occurrence of a nitrogen atom in oxidative state into a heterocyclic ring. Unlike other secondary metabolites, many alkaloids have independent biosynthetic pathways, may be originated from different amino acids (De Luca and Laflamme, 2001). With this, it is not surprising enzymes acting on alkaloid synthesis, while also performing functions in primary metabolism (Facchini, 2001).

The roles of the alkaloids in plants have been the subject of several types of research. It was believed that these substances are responsible for nitrogen secretion (like urea and uric acid in animals); however, this hypothesis is not accepted due to few concrete results. It is believed that most of the alkaloids present defense function in plants, due to their general toxicity their and ability to inhibit feeding (Wink, 1988).

Virtually all alkaloids are toxic to humans if ingested in sufficient quantities, however, many of these compounds are medicinally used, moving a worldwide volume of approximately 4 billion dollars (Julsing et al., 2006). Figure 5 shows some important alkaloids, such as morphine, berberine, vinblastine and scopolamine, which have medicinal properties and others such as cocaine, caffeine and nicotine, which have no medical use but is widely used as stimulants or sedatives (Facchini et al., 2004).

The alkaloid biosynthesis in plants requires the differentiation of specific cell types in response to specific environmental signals. For example, the N-methyltransferase enzyme, responsible for the chemical changes in the molecules of several nitrogenous compounds, has its activity increased by about 30% when etiolated plants of *Catharanthus roseus*, known as Vinca, were exposed too light (De Luca et al., 1988).

Few studies have shown specifically as changes in weather elements may affect the synthesis of this substance, but the most widely accepted hypothesis is that mainly light through phytochrome, modulates the regulation of protein and key enzymes involved in the metabolic pathways of these substances (Aerts and De Luca, 1992). Increases in alkaloids production of *C. roseus* were found with an increase of CO_2, mainly in a greater supply of nitrogen (Singh et al., 2015), indicating the relationship between plant metabolic responses and environmental factors.

Glycosides

Besides the alkaloids, glycosides also act as protective substances in plants. These substances can be divided into two types: cyanogenic glycosides, and glucosinolates. These metabolites are in wide varieties of plants. Alkaloids are not toxic in its natural state. For example, in crops such as sorghum and cassava, these substances are stored in the vacuole of the epidermal cells, whereas the enzymes responsible for the poisonous gas synthesis are in mesophyll (Poulton, 1990). When the plant is

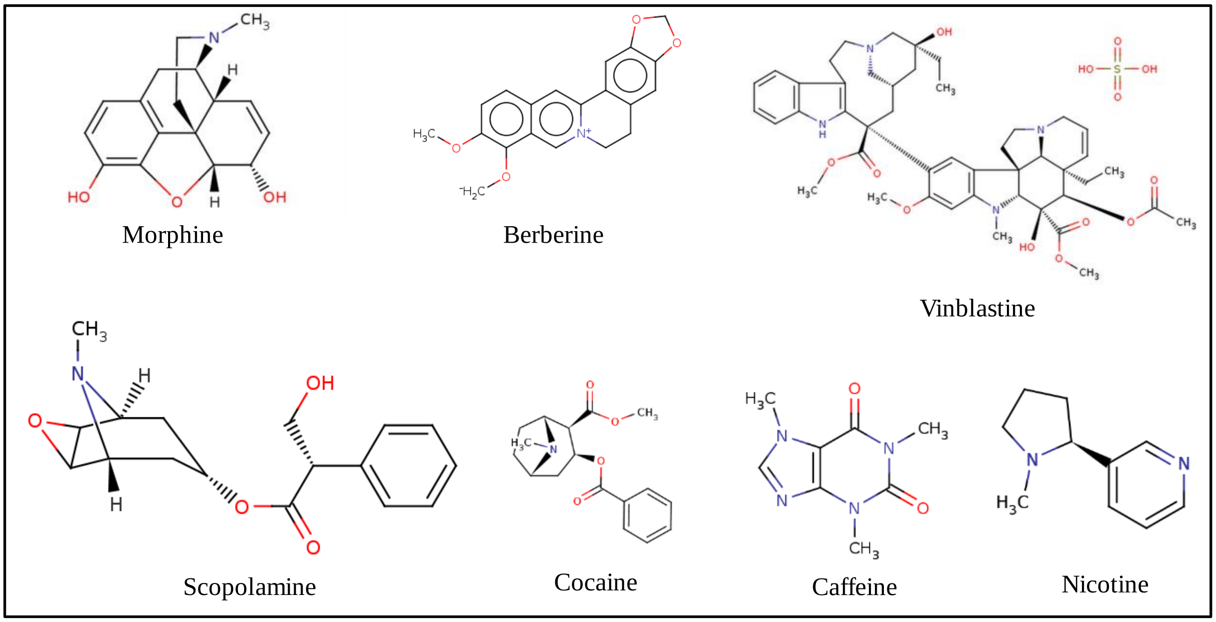

Figure 5. Some alkaloids of economic importance used by humans.

physically injured, cyanogenic glycosides (present in vacuole) are mixed, with hydrolytic enzymes (present in mesophyll), producing volatile poisons such as hydrogen cyanide (Taiz and Zeiger, 2010). Plant breeding programs aimed at reducing these compounds in crops of interest has been done. In cassava, for example, when fragments of CYP79D1 / D2 genes (responsible for the synthesis linamarin a cyanogenic glycoside) were silenced, it was observed the reduction of about 60-94% of these substances in leaves and surprisingly, 99% in roots (Siritunga and Sayre, 2004).

The influence of climatological variables in glycosidic compounds is significant. Studies in stevia, a small evergreen shrub with high sweetening capacity, has revealed that the transcript levels of 15 genes involved in vital pathways of steviol glycosides synthesis, were maximum at the temperature at 25ºC, while the transcription of 12 of the 15 genes was inhibited both at low temperatures (15°C) as at high temperatures (35°C). Most genes exhibited low transcription levels under water deficit, while the photoperiod did not significantly influence its transcriptions (Yang et al., 2015). These responses, however, cannot be generalized to all species, due to genetic specificity of each, and its adaptation to specific environments. This can be a stimulus for performing local research with cultures that have an economic interest.

Non-protein amino acids

Plants and animals incorporate the same 20 amino acids in their proteins. However, non-protein amino acids, which are not incorporated into proteins and roam freely, acting as protection substances in plants. Among some, may be cited the 2,4-diaminobutyric acid, 2,3-Diaminopropionic acid, L-3-Amino-2-(oxalylamino) propanoic acid and 2-amino-6n-oxalylureidopropionic acid (oxalylalbizziine) which are present in certain fodder and has been causing toxicity in ruminants (Mcsweeney et al., 2008).

Diaminobutyric acid can act as a competitive inhibitor of gamma-aminobutyric acid (GABA), an important regulator of neuronal excitability. A study with synaptosomal fraction isolated from mouse brains has revealed that diaminobutyric acid causes a competitive inhibition with the site of GABA absorption in a short time; for more prolonged periods, this inhibition was not competitive, being the effectiveness of this inhibitor, dependent on sodium levels and the temperature sensitive. (Simon and Martin, 1973).

Final considerations

The evidence presented here indicates that the synthesis and inhibition of plant’s secondary metabolites are dependent upon its primary metabolism, and are subject to great influence by environmental factors, primarily due changes in the transcription factors responsible for its synthesis. Environmental factors such as extreme temperatures, radiation, CO2 levels and water availability are presented as potential factors in changing the pathways of several secondary metabolites in plants. The same can be said for biotic factors such as plant damage caused by pathogens or herbivores. The plants have

Table 1. Limitations and challenges to be achieved upon the control of secondary metabolite synthesis in plants.

Limitations	Challenges
Terpenes	
Dynamic and complex synthesis of terpenes in response to environmental factors in important crops such as watermelon, Gac, papaya and tomato are still limited.	To identify the pathways responsible for synthesis of these compounds that are influenced by environmental factors.
Reduction in endogenous concentrations of JA under increased CO_2 levels.	Obtaining superior genotypes with high JA synthesis in presence of elevated CO_2 levels.
Phenolic compounds	
Multiple-gene regulation of PAL enzyme's activity.	To identify PAL genes in important crops responsible for role in the defense responses, under different environmental stimuli.
Information about how the environmental factors acts on coumarins' synthesis are still limited.	To reveal the pathways modified by environmental factors aimed at production of responsive plants with greater therapeutic importance.
Little information about how the environmental factors acts on Lignin's synthesis and the response to lignification related to presence of peroxides.	To identify how environmental factors act on its synthesis or inhibition, aiming to obtain superior genotypes with greater or lesser lignin content.
The pleiotropy for miRNA156, controlling the synthesis of anthocyanin and flavonoids and its relationship with SPL9, a key regulator of plant development which promotes flowering in Arabidopsis.	To identify the kind of pleiotropy acting in this crop, and to perform studies with economical-important crops in order to clarify the genome-wide significant associations with plant complex traits.
The complex interaction between soil and environmental factors on the synthesis of anthocyanin.	To create genotypes with high anthocyanin production and acclimatized in varied environments.
Hyper sensibility to UVB radiation in plants mutants or with little synthesis of flavones and flavonols.	To increase endogenous concentration of flavones and flavonols in crops in order to mitigate the effects of the reduction of ozone layer.
Little information about the interaction of biotic and abiotic stimuli on Isoflavones synthesis and plant's resistance.	To identify the pathways altered by environmental factors in order to produce more resistant plants.
Nitrogen compounds	
Research evaluating the influence of environmental factors on alkaloids' synthesis are still limited.	Due its medicinal importance, is highly encouraged the realization of research aiming at evaluating the influence of environmental factor in its synthesis.
Limited information about dynamic of cyanogenic glycosides in plants such as cassava and sweet potato.	To use genomic tolls in order to silence genes responsible for glycosides' synthesis in economical-important crops.
Toxicity in ruminants caused for non-protein amino-acids.	To balance the lesser concentration of toxic compounds such non-protein amino acids with the maintenance of plants' defense against herbivores.

created protection strategies which allowed them higher levels of evolutionary fitness. Scientific advances in molecular biology over the past years have been useful in understanding the dynamics of secondary metabolite synthesis in plants. Recent techniques in gene editing have been useful and can be used by breeders aiming to exert greater control over the expression or inhibition of these substances. A synthesis of limitations and the challenges to be achieved upon the control of secondary metabolite synthesis in plants is shown in Table 1.

Conflict of Interests

The authors have not declared any conflict of interests.

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Review

The utilization of tropical legumes to provide nitrogen to pastures: A review

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Sustainable models of animal production constantly seek for ways to pasture production with inputs reduction. In this context, the introduction of forage legumes in the pasture system is fundamental to fix nitrogen from the atmosphere and supply it to grasses, increasing its production and persistence, enhancing animal nutrition as it grazes, at the same time. Still, the use of plant mixes in the system brings further benefits to it, such as better utilization of fertilizers as well as enhances the nutrient cycle and soil coverage. However, there are still many gaps in our knowledge regarding intercropping legumes and grasses, especially the use of tropical legumes. The purpose of this review is compiles data about forage legumes as well as analyses them to find new tendencies and gaps on the knowledge to shed some lights on researches in this area. We also expect that this review will help researchers and producers to understand the role of forage legumes in a pasture system and that there will be an increase in its utilization worldwide, especially in the tropics, where nitrogen is easily leached and lost from the pastures. With emphasis on description of promising tropical legumes but still little used *Arachis pintoi*, *Macrotyloma* and *Neonotonia whightii*.

Key words: *Arachis pintoi*, grass, *Macrotyloma axillare*, mixed pasture, *Neonotonia wightii*.

INTRODUCTION

Since 1996 there have been an increment in animal productivity (production by area unit) in milk as well as in meet and other products in tropical pasture systems without the respective increase in the pasture area (IBGE, 2016). In Brazil the total pasture area was actually reduced by 3% in the last 10 years, and is estimated to be around 172 million hectares in 2006 (IBGE, 2016). This reduction was accomplished by improving the pastures from its establishment, managing and nutrient reposition, resulting in an increase of the use of fertilizers,

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especially synthetic nitrogen. However the volume of fertilizers used in pasture in Brazil is still low and estimated to be around 3.6 kg/ha.year^{-1} of NPK formula (Barcellos et al., 2008).

Nitrogen, in modern agriculture, is used in large quantities (ANDA, 2016) and it represents the most expensive nutrient applied in crops (Cantarella, 2007). It is estimated that the use of nitrogen fertilizers warrants the livelihood of 40% of the global population, which it would not be possible without its use (Mosier and Galloway, 2005).

Amongst the macronutrients, nitrogen is the most important to maintain plant production, mainly in monoculture of grass pastures, because it works in protein synthesis, which in turn will be used in several metabolic processes in the plants, justifying the use of heavy amounts of this element (Raij, 2011). Therefore, when there is low availability of this nutrient to the plants, the process of pasture degradation is started. Degradation brings great negative consequences to the production system, such as the lost of vigor and the productivity of the forage, reflecting in the capacity of support of the affected areas, leaving them susceptible to undesired effects, such as the attack of insects and the emergence of weeds (Nascimento Junior et al., 1994).

In order to reduce the dependence on synthetic nitrogen in pasture systems there is a search for alternatives to provide this nutrient to the grasses. The most promising at this moment is the use of legumes as forage, which increases the nitrogen in the system of biological fixation of nitrogen (FBN) in symbiosis with bacteria that have an enzyme (nitrogenase), usually from the *Rhizobium* and *Bradyrizobium* genera. The FBN is the transformation of N_2 in NH_3 and after that in reactive organic forms (Cantarella, 2007). Among the forage legumes to use in pastures and used most commonly in Brazil are the genera *Arachis, Sthylosanthes*, *Neonotonia* and *Leucena*, as well as the promising *Macrotyloma*.

THE IMPORTANCE OF NITROGEN IN PASTURES

The production of grass fed bovines, due its low cost and practicality is the most common system of animal production in the world (Castagnara et al., 2011). There is the need to apply fertilizers to replace nitrogen in order to maintain or increase production in tamed pastures exclusively with grasses (Mesquita et al., 2010; Da Silva et al., 2010; Gimenes et al., 2011). This is due to the fact that nitrogen is part of several organic composts, amino acids and nucleic acides, and is necessary in higher quantities than any other nutrient by the plabts (Epstein and Bloom, 2006).

Nitrogen exists in the soil mostly in its organic form (more than 95% of the total N) and its inorganic fractions may occur as NH_4^+ and NO_3^- (Cantarella, 2007). Tropical soils are generally characterized for low pH, with influences directly the absorption of nitrogen by the plant roots, resulting in a lower intake of this nutrient by the plants.

The absorption of nitrates is greater in lower pH, while the intake of ammonia is greater when pH is near to neutrality (Costa et al., 2006). Therefore, liming influences and stimulates in many soils the growth of the root system and, in doing so, improves the utilization of the available N.

Soil utilization is a factor that acts directly on the way that crops respond to N fertilization, meaning that where soils were cultivated for just a few years or were kept in rest for a while there will be less response to N fertilization (Raij, 2011). The reason for it is that it may be occurring mineralization of the organic matter which in turn provides N to the plants.

Furthermore, it is worthy to note that it is also necessary environmental conditions, such as temperature, humidity, luminosity and nutrients availability in order for the grasses thrives in a specific area. Among these conditions, in tropical regions, the nutrient availability is one of the factors that interfere the most in productivity, mainly due the natural acidity of the soils.

If the availability of N is low for a long period of time, there is going to start a degradation process. This is because the plants that show symptoms of N deficiency have their photosynthetic rate diminished due to the yellowish color of its older leaves, which in turn reduces the plant growth, with less productivity. The low availability of N may also affect the root development, the photosynthesis and the production of photoassimilates, as well as the growth rate of leaves and roots (Taiz and Zeiger, 2004).

Usually N deficiency happens in pastures that do not receive N fertilization or received a lower dose than the recommended, as the availability of N via mineralization of organic matter it is not enough to supply the grasses demand. Therefore, it must be supplemented by N fertilization (Guilherme et al., 1995). Werner et al. (1996) recommend as a minimum to apply 40 to 80 kg/ha year of N to maintain the pastures, depending on the forage being managed.

However, the responses to N fertilization over the accumulation of forage mass depends on the potential of forage production, the relationship between the photosynthetic rate and the concentration of N, and the expansion and spatial distribution of the leaves in the pasture canopy and its impact in the light interception (Gastal and Lemaire, 2002). The nitrogen, because of its active participation in the synthesis of organic compounds, may change the structural characteristics of the plants, such as the size of the leaves, and tiller density, as well as its morphogenic characteristics, such as the rate of leaf appearance and leaf senescence (Lemaire and Chapmam, 1996; Lemaire et al., 2011).

Grass tillering is influenced by N fertilization (Jewis, 1972; Hodgson, 1990; Chapman and Lemaire, 1993). N fertilization has a direct effect over the tiller population and its density also in tropical grasses, which is determinant to forage biomass production in pastures, according to Alexandrino et al. (2004), Fagundes et al. (2005), Pates et al.(2007), that evaluated *Brachiaria brizanta* cv. Marandu, *Brachiaria decumbens* cv. Basiisk and *Panicum maximum* cv. Tanzania, respectively. Thus the number of leaves in a plant increases as the number of tillers also increases., which is an important characteristic for establishing forage productivity. However, de Fialho et al. (2012) did not find significant differences between pastures that were fertilized with N with doses varying from 50 to 200 kg/ha.year, probably due the phenotypical plasticity of the species *B. brizantha* cv. Marandu.

Other than increasing tiller population and density, N fertilization increases the ratio of young tillers in the pasture, as the nitrogen speeds the appearance and death of tillers, increasing directly the tiller's rate of senescence, due to its continuous renovation. If N fertilization is used, the forage must be harvested more frequently so the senescent material does not accumulate and the nutritive value of the forage does not decrease (Paiva et al., 2011).

The rate of leaf appearance, defined as the number of leaves that appear per tiller during a certain period of time, it is central to the plant morphogenesis, because it may change the size of the leaves, the tiller's density and the number of leaves per tiller (Lemaire and Chapman, 1996) and also suffers the influence of N fertilization, as the N fertilization stimulates plant growth, bringing as a consequence the internodes elongation, which makes the leaf to be expelled from the sheath of the previous leaf, thus increasing the rate of leaf appearance (Oliveira et al., 2007).

There are some words to describe the leaf appearance and among them there is the term "phyllochron", defined as the interval between the appearance of two successive leaves. Phyllochrons may be reduced with N fertilization because it increases the plant growth, resulting in bigger regrowth capacity, given to the plants a fast replacement of leaf area (Lemaire and Chapman, 1996).

Cassol et al. (2011) observed that with the utilization of nitrogen there was a greater amount of leaves produced by oats in 45 days than in 60 days without its use, noted that if N fertilization was not used in oats (*Avena strigosa*) and ryegrass (*Lollium multiflorum*) the plants needed 15 more days to accumulate a similar amount of dry matter than when N fertilization was used. This proves the influence of nitrogen in the production of citocinine, an hormone responsible for the plant growth and that acts activating of the process of cellular division and differentiation (Marschner, 1995). Nitrogen fertilization also helps with the activation of the meristematic tissues in plants, reducing the number of dormant gems and promoting maximum tilling in grasses (Jewiss, 1972).

Providing N to the plants increases the dry matter production in temperate grass pastures, such as perennial ryegrass (Quatrin et al., 2015) and also in tropical forages, as reported for guineagrass (*Panicum maximum* IPR-86 Milenio) (Sarmento et al., 2005), brachiaria palisadegrass (*Brachiaria decumbens* cv. Ipean) (Fagundes et al., 2005), Marandu palisadegrass under continuous stocking (Mesquita et al., 2010) and rotational stocking (Gimenes et al., 2011; Costa et al., 2016). Taffarel et al. (2016) observed that the N fertilization increased dry matter production because altered the structural characteristics of the plants, such as a higher canopy, longer stems, higher leaf elongation. Costa et al. (2016) founded that topdressing N fertilization in Marandu palisadegrass increases dry matter production and N-accumulation in plant tissues.

FORAGE LEGUMES AS A NITROGEN SUPPLIER

Monoculture of forage species was used for many years in pastures. However most of this areas are now degraded or in some stage of degradation, due mainly to mismanagement and/or for lack of nutrient reposition, especially because of low N availability (Dias-Filho, 2014). In Brazil, country with roughly 172 million hectares of pastures (IBGE, 2016), the problem of degradation in pastures is very significant. Based on the total area of pastures in Brazil, according to official data (IBGE, 2016), it would be possible to estimate that around 100 million hectares of pastures in that country would be subject to some sort of remediation because these areas would be in a moderate to high level of degradation.

Even though there is N deficiency in pasture systems, nitrogen is the most abundant element in the atmosphere, with approximately 80% on its composition. However it is found in a form that cannot be captured and metabolized by plants (N_2). It can be absorbed by most of the plants in the form of NH_4^+ or NO_3^- that are compounds more reactive and present in the soil. In legumes, that are plants of the Fabaceae or Leguminosae the nitrogen present in the air in its N_2 form is transformed by the enzime nitrogenase into NH_3 by bacteria that receive energy from the plants and transfer nitrogen in turn to them. Plants spend energy in this process but obtain, as an advantage, nitrogen, even if the soils are poor on it (Taiz and Zeiger, 2004). Biological nitrogen fixation is the process that changes inert N_2 to biologically useful NH_3. After incorporation of N in the legume plant tissue, its plant remains will go to the soil and become organic matter, where N is mineralized to the forms NH_3 and NH_4 that can be absorbed by the grasses (Raij, 2011).

In the industry, for every kg of N produced, 15 Mcal of energy is spent to produce it, energy that is coming from fossil fuel, which is not sustainable and is dependant in oil (Resende et al., 2003). As the price of fossil fuel skyrocket in the 70's, the research regarding biological fixation of nitrogen (FBN) was stimulated (Serraj, 2004).

FBN has an important role supplying N to agricultural systems as is estimated that FBN contributes with 32.0 Tg/year of N, or around 30% of the N produced artificially as a fertilizer. In Brazil the amount of N supplied by FBN is around 7.3 Tg/ano^{-} which is almost three times the amount produced by the industry (Cantarella, 2007).

Diehl et al. (2013) said that mixed systems with grasses and legumes provide a better utilization of the pastures all year round, presenting also more forage mass production and a higher stocking rate. Furthermore, areas that were cultivated with legumes have an effect on the next crops (Mesquita et al., 2015; Alves et al., 2016) as the legumes capture and release N into the system.

The introduction of legumes in pastures that have only grasses is a sustainable alternative and rarely used. It may help improve soil quality, reduce N fertilization and bring ecological benefits to the system production, as well as improve the quality of the animal diet (Barcellos et al., 2008).

The reduction of the use of nitrogen fertilizers in pasture systems via the implementation of legume forages have a direct effect in the reduction of the emission of greenhouse gases and pollutants, as well as reduce the impacts of the eutrophication in water bodies, because it lowers the lixiviation of nitrates, thus lowering the cost of production. However it is important to notice that the mix grass x legume must be adaptable and compatible with local conditions, as well as resist to insects and diseases, in order to be persistent and permanent (Valle and Zimmer, 2013).

The legumes seedlings emerge from the soil without any association between roots and rhizobia and may never have the association until the end of its cycle (Epstein and Bloom, 2006). These same authors also relate that in conditions where N is limited the symbionts reach out one to each other, via signal exchange, which results in the process of infection and development of the nodules that will capture nitrogen. The most common symbiosis occurs between legumes and bacteria known as rhizobia, which includes the *Rhizobium* and *Bradyrhizobium* genera.

The FBN occurs through the symbiosis between the microorganisms that exists in the soil and the plant legumes, contributing to the self-sufficiency of all plants (legumes and grasses) of the mixed pasture as the source of nitrogen, The legumes transfer nitrogen to the grasses of the system through the decay of its vegetative material that fall in the soil, exudates and through the roots. Carranca et al. (2015) noticed that visible roots and nodules of the legume European yellow lupine (*Lupinus luteus* L.) and subterranean clover (*Trifolium subterraneum*) may contain from 7 to 11% of the total N fixed by the plant.

Thus, the release of the N fixed in the soil will supply the needs of the legume and the grass established. This association may incorporate up to 500 kg/ha.year of N (Siqueira and Franco, 1988). However, data from tropical legumes show lower quantities of N fixed per year. Giller (2001) in his book reports that FBN in tropical legume species such as *Arachis pintoi* may fix from 1 to 7 kg/ha of N in a 12 week period of evaluation in Colombia (estimated 4 to 21 kg/ha.year); *Calopogonium mucunoides* supplied from 136 to 182 kg/ha.year and 64 kg/ha.ano in the Samoa Island and in Brazil, respectively; for *Stylosanthes spp.* cultivated in Australia the N fixed was 39 kg/ha in a 17 weeks period (estimated 110 kg/ha.year).

The lower values for N fixed per year in tropical regions, when compared to the potential fixation, may be associated to the higher exigency level for nutrients by legumes versus tropical grasses (Werner et al., 1996), not always met by the soil reserves, often acids and low in phosphorus, calcium and micro nutrients. Another difficulty for increasing N fixation is the fact that legumes are plants that have a C3 carbon metabolism, whereas tropical grasses are plants with a C4 cabon metabolism and ideal temperature around 35°C, which makes tropical grasses more aggressive in competition for water and efficient in its utilization of nitrogen (Corsi and Nascimento Junior, 1984). These two limitations for the complete development of forage legumes in mixed systems of tropical pastures help to explain the low persistence of the legumes in these systems and demonstrate the great challenges that must be surpassed by research.

Spain and Pereira (1985) estimated that the ideal ratio of legumes in consortium with grasses in temperate pastures is from 20 to 40% of the forage mass expressed in dry matter of the pasture, in line with information from Thomas (1992) that estimated the ideal ratio above 20 to 30% of total mass of forage. Saia et al. (2016) researching several temperate legumes and ryegrass reported the presence of legumes in the mix varying from 30% (*Trifolium resupinatum* L.) to 69% (*Trigonella foenum-graecum* L.) of the total forage mass. Aguirre et al. (2014) reported problems with excessive participation of common vetch (*Vicia sativa* L.) during the winter (56% of the forage mass), reducing in the summer to 8.8% in a mix with Coastcross grass (*Cynodon dactylon* L. Pers. Cv. Coastcross-1), while arrowleaf clover participated with 17.6 to 5.8% of the forage mass also in mixed pasture with Coastcross.

There are no specific studies to estimate the ideal range of participation of the legumes in the forage mass in tropical grasses and legumes in mixed pastures. However the presence of legumes even below 20% has shown an increase in the forage mass and also in the nutritive value of the pasture. Pinheiro et al. (2015) reported that pastures with 14 to 17% of Brazilian stylo Campo Grande (a mix of 80% *Stilozanthes capitata* and 20% *S. macrocephala*) in the composition of the forage mass in a mix with Tanzania guineagrass (*Panicum* *maximum* cv. Jacq. Tanzania 1) produced forage equivalent to a pasture fertilized with 75 and 150 kg/ha of N.

Martuscello et al. (2011) compared the dry matter production of Brachiaria decumbens with Estilozantes cv. Mineirao (*Stylosanthes guianenses*), Calopo (*Calopogonium mucunoides*) and a pasture fertilized with 50 or 100 kg/ha of N and concluded that the dry matter production of the mix with Brazilian stylo was similar to the pastures fertilized with 100 kg/ha of N, while the mixed pasture with Calopo did not differ of a pasture without fertilization with N. However, there is no mention in this research of the ratio of the legume forage mass and total mass, which cannot explain if the presence of Calopo was enough to be effective in the total mass of forage in the pasture.

On the other hand, in a experiment conducted in the Instituto de Zootecnia, in Nova Odessa, Brazil, where legumes were implanted in separate plots in pastures already established with Aruana guineagrass (*Panicum maximum* cv. Aruana) for 5 years, Calopo was the legume that persisted in the pasture with the lowest ratio, when compared with other legumes, with a total forage mass of 13.3%, and, as a result, the protein brute in this mixed pasture was similar to pastures with a monoculture of Aruana guineagrass (Gerdes et al., 2009), which indicates that to increase the presence of Calopo in established pastures more research must be done.

Even if a reduction of the presence of forage legumes, or its complete extinction from the system, occurs, there will be a persistence in the N cycling and there will be some benefits for the mixed pasture. According to Menezes et al. (2015) would be necessary from 130 to 150 kg/ha of N to maintain the pasture productivity in areas with Xaraes palisadegrass two years after the disappearance of Brazilian stylo that exists in that pasture before, at the ratio of 34 and 52%. Also in this research Alves et al. (2016) evaluated the production and morphogenic characteristics of Xaraes grass and concluded that there was a residual effect from the incorporated nitrogen to the system by the legumes.

The amount of N incorporated by legumes in pasture systems range from 75 to 150 kg/ha.year of N (Martuscello et al., 2011; Menezes et al., 2015; Pinheiro et al., 2015) and are very significant to the maintenance of pasture productivity, avoiding the degradation of these systems, justifying financially environmentally researches to use forage legumes in mixed pastures.

CHARACTERÍSTICS OF SOME PROMISING LEGUMES TO USE IN TROPICAL REGIONS

The utilization of legumes as a forage is a promising alternative in the search for sustainability in several types of climate and soils. Brazil is considered a continental country and has several biomes bringing with them variations on climate, soils and its natural acidity. Acidity is one of the main factors that may affect the establishment of legumes. However some tropical forage legumes are highly adaptable to soils with low fertility and acids (Barcellos et al., 2008; Andrade et al., 2015).

By the end of the 80’s several were the studies with legumes and many of the plants shown potential and were efficient. Even showing potential, many of them are still unexplored due to low initial growth and lack of persistence of some species, recognized as the most limiting characteristic impeding its use.

Currently the cultivars of forage legumes that have being more studied and, thus, have more information compiled are: Brazilian stylo (*Stylosanthes spp.*), forage peanut (*Arachis pintoi*) and leadtree (*Leucaena spp.*), as they are more cultivated and/or promising.

There is an attempt, on this review, to discuss legumes that have a high potential to be used in mixed pastures with grasses but they are still underused by rural producers, like *Neonotonia wigthii* (perennial soybean), *Macrotyloma axillare* (perennial horse gram). Among them, there is *Arachis pintoi* (forage peanut), because even though there are several studies about this genotype it is not well utilized by the producers, probably because the need to reproduce them by seedlings which makes it more expensive and difficult to disseminate.

Perennial soybean (*Neonotonia wightii*)

Perennial soybean is originally from Africa and is also found in Southwest Asia (Tang et al., 1987). Perennial soybean is one of the most known forage legume in Brazil, due to its adaptability to the local climate changes. It was introduced in Brazil by the Instituto Agronomico de Campinas in 1956 (Alcantara and Mattos, 1976). It needs fertile soils, with pH around 6.0 and it is exigent in phosphorus as some other legumes are. It is considered by Barcellos et al. (2008) as a possible invasive in areas of crop-pasture integration because it benefits from the fertilizations done to the crops.

It is a perennial legume, herbaceous, prostrated, voluble, with trifoliate, oval and elliptical leaves and deep root system (Veasey et al., 1993). In ideal conditions this plant can fix from 40 to 140 kg/ha.year of nitrogen, if its nutritional needs are met and the pH is in an adequate range (Carvalho, 1986). Werner et al. (1996) classify this legume in the Group I of exigencies in soil fertility, recommending a soil base saturation to its implementation around 70 and 60% to its maintenance.

Barcellos et al. (2008) highlight the species low tolerance to overgrazing which is consistent with its growth behavior (voluble) and its good natural re-seeding. Perennial soybean (*N. wightii* Verde cv. Tinaroo) as a monoculture in pastures shown to be very promising as animal fodder and resulted in higher weight gain in beef cattle (1,375 kg/ha of weight gain) then Green Panic grass (*Panicum maximum* var. Trichoglume cv. Petrie) fertilized with nitrogen (75 kg/ha.year of N) (1,047 kg/ha of weight gain) in an experiment conducted for 4 years (Lourenço et al., 1998).

Before they started this experiment, grasses and legume had being seeded in together. However an insect attack (spittlebug) on the Green Panic helped the perennial soybean to dominated the pasture and it becomes exclusive, indicating a great potential to be planted in such with other grasses (Lourenço et al., 1998). The authors note that the pastures were fertilized before the experimental period started with phosphorus (100 kg/ha P_2O_5) and potash (60 kg/ha K_2O) and the pastures that had only legumes were fertilized with micro nutrients (5 kg/ha of copper sulphate, 5 kg/ha of zinc sulphate and 200 g/ha of sodium molybdate), which may be one of the causes to the high persistence and animal productivity of the perennial soybean-exclusive pasture.

Gerdes et al. (2009) reported that when perennial soybean (*N. wightii* NO 2348) was seeded in Aruana guineagrass pastures five years old it reached 42.2% of the total forage mass one year after its seeding when grazed by sheep, standing higher than the 20% recommended by Spain and Pereira (1985) as a minimum ratio. There was a significant transfer of nitrogen from the legume to the grass, which increased the protein brute of the grass to 10.9% when compared to pasture of exclusively Aruana guineagrass (9.3% PB). It was emphasized that P and K was supplied, as well as micro nutrients, which are important to meet the nutritional needs of the forage legumes.

The studies with *N. wightii* have being rescued by group of researchers in order to increase the knowledge about this plant so it can be introduced in new mixed pasture. The results obtained in hothouses evaluating the access of perennial soybean *N. wightii* NO 278 described its implementation capacity (Silva et al., 2016a) and the morphogenesis of its development using the elongation rate of its leaflets in length and width in its initial phase (Silva et al., 2016c).

Perennial horse gram (*M. axillare*)

Another option as a promising forage legume is *M. axillare* that was originally from tropical Africa (Bogdan, 1977) and it was introduced in Brazil in the middle of the 60's when a collection was sent to the Instituto de Zootecnia (Rocha, 1988; Paulino et al., 2008). It is a perennial plant, herbaceous, climber, voluble with branches finely pubescent and trifoliate leaves (Bufarah et al., 1981).

This legume has important characteristics to adequate itself in a pasture system, especially in regions where the soils are deficient in nutrients; it is fast growing and has good tolerance to low fertility soils, as already noticed by Werner et al. (1996) when it was classified in the group with a lower exigency among the forage legumes, needing a soil base saturation around 50%. Furthermore its persistence is high under grazing, because it has its growing favored for a low acceptance by the cattle, due to its bitter flavor due to its tannin content (Barnes, 1996).

Matos and Pedreira (1984) observed that this species has good growth along the different seasons of the year, which is a very important factor to productivity and persistence of the forage plant. Also in some recent studies Silva et al. (2016b) described the establishment of the perennial horse gram access and the morphogenesis of its development through the elongation rate of the leaflets and its length and width during the initial phase (Silva et al., 2016c).

Perennial horse gram access *M. axillare* NO 279 did not shown differences in its development with increasing doses of calcium (Barbosa, 2016), corroborating the information from Werner et al. (1996), which was its good tolerance to acid soils and with low base saturation. On the other hand, increasing phosphorus doses increased the dry matter mass production of the shoots, roots, nodules, leaf area, leaf numbers, branch numbers and nodule numbers (Barbosa, 2016) indicating the need for supplying phosphorus even in soils with median level of this nutrient (above 15 mg/dm^3 described by Werner et al., 1996).

Gerdes et al (2009) in an experiment already described for perennial soybean observed a good stand of *M. axillare* NO 279 in Aruana guineagrass pastures, with the legume reaching up to 43.2% of the forage mass after one year of the seeding and the protein brute of Aruana guineagrass reaching 11.9% among all mixed pastures.

Pinto peanut (*A. pintoi*)

A. pintoi is a native plant from the Cerrados of Brazil, adapted to acid soils and low fertility. The cultivar Belmonte is prominent in tropical regions because of its high nutritive value, high persistence and tolerance to shade, showing that it is apt to mixed pastures with grasses (Barcellos et al., 2008), *A. pintoi* cv. Belmonte is an herbaceous plant with stolons that is vegetative propagation (Paganella and Valls, 2002).

The genus *Arachis* comprises plants adapted to tropical and subtropical conditions with uniform rainfall and a dry season not superior to four months (Ramos et al., 2010). As per its persistence, *A. pintoi* cv. Belmonte have characteristics such as a prostrated growth behavior and it is stolonifera (which protects the plant from grazing), long life cycle and tolerance to shade (Andrade et al., 2006).

Assis et al. (2008) evaluated 21 genotypes of pinto peanut and observed that the dry matter production varied from 1,609 to 4,132 kg/ha.year, and the Belmonte cultivar was a highlight among all genotypes. It is worthy to note that the mix between Belmonte cultivar and the forage grasses has increased the productivity and profitability of the systems where it is already in use (Andrade et al., 2012; Oliveira, 2007).

Fialho (2015) studying the characteristics of *A. pintoi* cv. Belmonte under continuous stocking observed that the grazing intensity increases the leaf appearance rate, thus given evidence that this plant is capable of adjusting its growth at different defoliation rates. Furthermore, the results indicate a consistency in the leaf area index, which indicates that the Belmonte cultivar has a higher adaptation capability, which may be useful as it gives more flexibility to be used and managed.

Conflicts of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Optimal sample size and data arrangement method in estimating correlation matrices with lesser collinearity: A statistical focus in maize breeding

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Information about data arrangement methodologies and optimal sample size in estimating the Pearson correlation coefficient (*r*) among maize traits are still limited. Furthermore, some data arrangement methodologies currently used may be increasing multicollinearity in multiple regression analysis. This study aimed to investigate the statistical behavior of the *r* and the multicollinearity of correlation matrices among maize traits in different data arrangement scenarios and different sample sizes. Data from 45 treatments [15 simple maize hybrids (*Zea mays* L.) conducted in three locations] were used. Eleven traits were accessed and three datasets (scenarios) were formed: (1) Coming from all the sampled observations (plants), *n* = 900; (2) Coming from the average of five plants per plot, *n* = 180; and (3) Coming from the average of treatments, *n* = 45. A thousand estimates of *r* were held in each scenario to 60 sample sizes by bootstrap simulations with replacement. Confidence intervals (CI) were estimated. One hundred eighty correlation matrices were estimated and the condition number (CN) calculated. Data coming from average values of plots and average values of treatments overestimates the *r* up to 24 and 34%, resulting in an increase of 24 and 131% in the matrices' CN. Trait pairs with high *r* require a smaller number of plants, being the CI inversely proportional to the magnitude of the *r*. Two hundred and ten plants are sufficient to estimate the *r* in the CI of 95% < 0.30.

Key words: Average values, bootstrap, confidence intervals, sample tracking, *Zea mays* L.

INTRODUCTION

One of the most used statistical methods to measure the degree of association (linear) between two random traits is the Pearson product-moment correlation coefficient (*r*) (Pearson, 1920) and has been used in ecological studies to estimate the direction and degree of association among traits (Annicchiarico et al., 1999; Yao and

Mehlenbacher, 2000; Yang and Su, 2016).

As this measure only reveals the linear association between two traits, techniques such as path analysis (Wright, 1923) and canonical correlation (Hotelling, 1936) were developed in order to explain the interrelationships among traits or group of traits, being worldwide used in plant breeding. These techniques depend on the linear correlation matrix among traits and, due its estimates be based on principles of multiple regression, the low dependence among the traits considered as explanatory is required. When this assumption is not met, it is said that the matrix presents multicollinearity (Blalock, 1963).

Although there are techniques to adjust the multicollinearity (Hoerl and Kennard, 1970b) these techniques are essentially correctives, applied only after the linear correlation matrix be estimated. Since the estimates of correlation coefficients basically involve the behavior analyses of the variances, that is, deviations from the average, it is possible that some methods of data arrangement currently used may be masking the actual averages and variances of a trait (*X*) on a dataset of (*n*) observations. For example, in a bibliographic survey, we found that the correlation matrices of some agronomic studies using path analysis were estimated with average values of several plants sampled in each experimental unit (Khameneh et al., 2012; Toebe and Cargnelutti 2013; Adesoji et al., 2015; Kumar and Babu, 2015; Nataraj et al., 2015).

In field experiments, it is very common to access values of traits in several plants of each experimental unit. The utilization of average value of these plants in order to estimate the *r* and perform inferences to the population under study, however, may be questionable. In a theoretical explanation focused on plant breeding, Olivoto et al. (2016) reported that the use of average values in estimating the *r* between a traits pair (e.g. $r_{x:y}$) may overestimate its magnitude mainly due the reduction of standard deviation (SD) in the dataset, when compared with estimates performed with values coming from all sampled plants. In addition, the observed SD (e.g. for *X* and *Y*) when average values of plots or treatments are used, represents the SD of the average of the originally sampled plants, and not the actual SD coming from all these plants; therefore, this SD is masked and tends to present lower itself. This fact should be taken into consideration, because the inference of the direction and magnitude of association among traits when average values are used is being made for a different population of the original.

There were no studies in the literature comparing different data arrangement methodologies on estimates of Pearson's correlation coefficients. In addition, the information about the optimal sample size in order to estimate the *r* among trait pairs in the maize crop in an acceptable confidence interval is needed. In this context, the aims of the present study were to (i) reveal the statistical behavior of estimated Pearson's correlation coefficients in different data arrangement scenarios and different sample sizes, (ii) reveal the impact of data arrangement scenarios and sample sizes on multicollinearity of matrices, and (iii) propose the optimal sample size in order to estimate *r* among trait pairs in the maize crop in an acceptable confidence interval.

MATERIALS AND METHODS

Site description and experimental design

Field trials were conducted in 2014/2015 growing season in Santo Expedito do Sul (27°56' S, 51°37' W; 728 m asl), São José do Ouro (27°44' S, 51°32' W; 796 m asl) and Viadutos (27°33' S, 52°00' W; 628 m asl), municipalities of Northeastern Rio Grande do Sul State, Brazil. During the experimental period, the air averages temperatures at the sites of the experiments were 24.5, 23.8 and 25.2ºC and the natural rainfall of 823, 958 and 746 mm, respectively. All locations are within a 70-km radius, have a Haplustox soil, and were chosen due to similarities of soil and climatic characteristics, which provided to them low variability of temperature and rainfall. Thus, abiotic effects on the plants' response were minimized as much as possible.

Prior to the installation of the trials, each site was surveyed for potentially disruptive characteristics. To ensure uniformity inside the block and heterogeneity between the blocks, a randomized complete block design in a 15 × 3 factorial treatment design (15 simple maize hybrids x three cropping fields) with four replications was used, totaling 180 plots. Each plot contained six 5-m-long cultivar rows, spaced by 0.45 m. Only the two central rows were used to prevent edge effects. In each plot, five representative plants (observations) were selected from which the ear was removed for further evaluation. To ensure that traits (of plant and ear) were assessed in the same individual, a sample tracking system was created, identifying each ear with a label containing a sequence number that characterized the site, the hybrid, the repetition and the evaluated plant.

Accessed traits

Plant height (PH) and the ear insertion height (EH) were measured (cm) from the ground surface to the flag leaf node and the support node of the highest ear at the stem, respectively. Tagged ears were evaluated at a laboratory. The following traits were accessed: ear length (EL) (cm), ear diameter (ED) (cm), number of rows per ear (NRE) (un), number of kernels per row (NKR) (un), cob length (CL) (cm), cob diameter (CD) (mm), cob diameter / ear diameter ratio (CD / ED) (decimal), total number of kernels per ear (TNK) (un) the thousand-kernel weight (TKW) (g). The ratings were performed as follows: The lengths and diameters were measured with a digital caliper. After counting the number of rows per ear and the number of kernels per row, the kernels of each ear were manually-threshed

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and cleaned with pressurized air. Subsequently, the kernels-weight was measured with an analytical balance and the total number of kernel each ear was measured with seed counter equipment. Finally, the grain moisture was measured with a universal moisture meter. With this data, and with the humidity adjusted to 14% base moisture, we determined the thousand-kernel weight each ear by the equation: TKW = [(KME/TNK) × 1000]. Where: TKW = Thousand kernel weight; KME = Kernel mass per ear; TNK = the total number of kernels per ear. All evaluations were carried out carefully in an ear at a time, to maintain traceability of the sample, avoid any systematic errors as well as minimize the random errors.

Statistical procedures

Bootstrap simulations

Three data arrangement scenarios were considered: (i) The data used were originated from all sampled observations (ASO), with a total sample size of 900; (ii) In this scenario, the data used were obtained from the average of the five sampled plants of each plot (AVP), with a total sample size of 180, and (iii) Finally, the average of the treatments (15 treatments × 3 locations), with a total sample size of 45 was considered (AVT).

Aiming to match the sample size in each scenario, 60 sample sizes (plants) were simulated. The size of the initial sample was 15 plants, and the rest were obtained with an increment of 15 plants up to 900 plants. For each one of 55 trait pairs [n × (n-1)]/2, where *n* =11, in each sample size of each scenario, 1000 simulations of the *r* were performed by bootstrap resampling with replacement (Efron, 1979). Thus, for each pair of traits, 1000 estimates of the *r* were obtained. Simulations were performed by the Structural Equation Modeling procedure in Statistica 10.0 software.

Descriptive analysis of correlation coefficients

In each sample size of each scenario, the 1000 simulated *r* were subjected to descriptive analysis, where it was determined the maximum, (97.5%), average, (2.5%) and minimum values. Later, the amplitude of the 95% confidence interval was calculated by the difference between the percentile 97.5 and 2.5%. For comparison, three trait pairs that came closest to the following *r* magnitudes were chosen: *r* ≈ |0|, *r* ≈ |0.5| and *r* ≈ |1.0|. The statistics mentioned of these three trait pairs has formed scatter diagrams where the x-axis corresponds to the number of plants and the *y*-axis corresponding to the descriptive statistics.

t-test to compare the correlation coefficient among the scenarios

In order to determine whether the inferences could be made with the average of 60 sample sizes, initially the *r* average of each traits pair at the different sample sizes were compared by *t*-test at 5% probability error (Steel et al., 1997) in the following scenario combinations: ASO × AVT, ASO × AVP and AVP × AVT. Inferences were made using the average of sample sizes for each pair of traits if the 60 samples presented the same result on the test.

A test comparing the 3300 values of *r* (55 trait pairs × 60 sample size) was also performed. Histograms were developed for each scenario combination (ASO × AVT, ASO × AVP and AVP × AVT) in order to show the behavior of the estimated *r* distribution. These procedures were performed using *t.test* and *hist* functions in R software (R core Team, 2016). Descriptive statistics such as asymmetry, average, mode, 25th and 75th percentiles, maximum, and minimum applied in each scenario are also presented in boxplot graphics. These procedures were performed using *summary* and *boxplot* functions in R software.

Diagnosis of multicollinearity in the scenarios

Data of 11 traits obtained by the average of 1000 bootstrap simulations in each sample size of each scenario were used in order to estimate correlation matrices. A total of 180 matrices (60 sample size x three scenarios) were estimated. In each matrix, multicollinearity diagnosis was performed by the condition number (CN) of the matrix. The CN was obtained by the ratio between the largest and the smallest eigenvalue of the matrix. The degree of multicollinearity was considered weak, moderate and severe when CN ≤ 100, between 100 and 1000 and ≥ 1000, respectively (Mansfield and Helms, 1982). A graph containing the number of plants (*x*-axis) and the CN of each scenario (*y*-axis) was developed. This analysis was performed using the Multicollinearity Diagnostic procedure in Genes software (Cruz, 2013).

RESULTS

Statistical properties of the correlation coefficient

The estimated *r* presented the largest amplitude when the lowest number of plants was used. For the pair EH x PH, the magnitude of *r* oscillates between -0.02 and 0.98 (Figure 1a), 0.42 to 0.99 (Figure 1b) and 0.71 to 0.99 (Figure 1c) in ASO, AVP, and AVT scenarios, respectively. This range was reduced as the number of plants increased; however, it appeared higher in the ASO scenario. The average *r* between the 60 different numbers of plants evaluated was increased by approximately 11% *(r* = 0.92) and 15% *(r* = 0.96), in AVP and AVT scenarios, respectively (Figure 1b and c).

For trait pairs with *r* ≈ | 0.5 | as NKR × ED, the amplitude of *r* was larger, irrespectively of the scenario and the number of assessed plants. With 15 plants, *r* ranged between -0.33 and 0.89 in the ASO scenario (Figure 1d), between -0.62 and 0.91 in the AVP scenario (Figure 1e) and between 0.03 and 0.90 in the AVT scenario (Figure 1f). The average *r* was increased by approximately 16% *(r* = 0.58) and 24% *(r* = 0.62), in AVP and AVT scenarios, respectively. Trait pairs with *r* ≈ | 0 | as DSDE x CE presented the highest amplitudes, with similar *r* distribution in the studied scenarios (Figure 1g to i).

For the pair PH × EH, 270 plants were enough to estimate the *r* in the ASO scenario in the CI 95% ≤ 0.10 (Figure 2a). For AVP and AVT scenarios, however, the number of plants needed was only 45 (Figure 2b) and 30 (Figure 2c), respectively. Trait pairs with *r* ≈ | 0.5 | (NKR x ED), needed 660, 465 and 285 plants, in ASO, AVP, and AVT scenarios, respectively. For CD/ED x EL combination, CI 95% ≤ 0.10 was not reached even with 900 plants.

Comparison of correlation pairs between the scenarios

The *t*-test revealed no differences among the sample sizes in all scenario combinations. Thus, the inferences

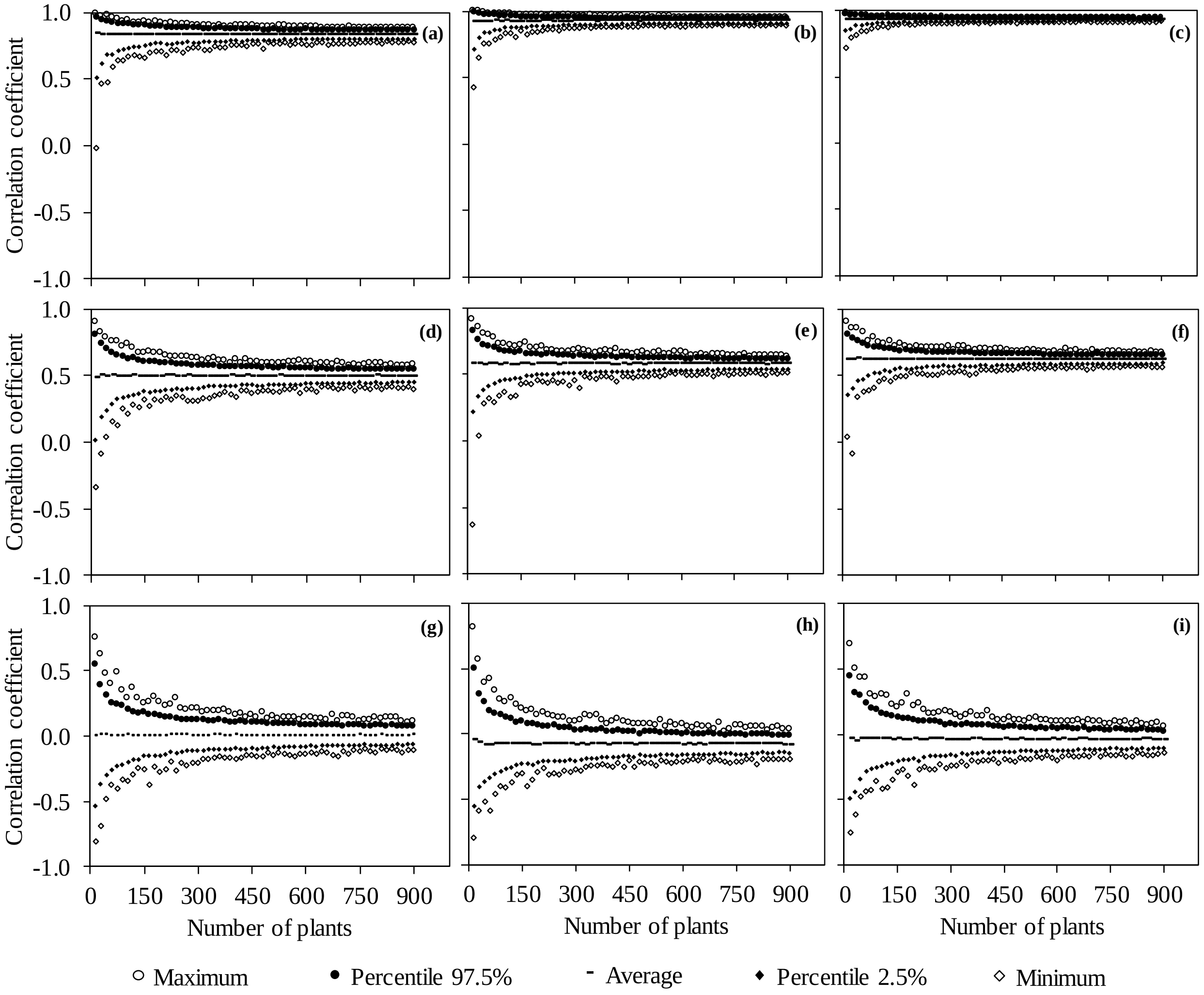

Figure 1. Descriptive analysis of 1000 bootstrap estimates of Pearson's correlation coefficient. Symbols represent the maximum values, percentile 97.5%, average, percentile 2.5% and minimum, obtained for the pair of traits plant height x ear height estimated in ASO (a) in AVP (b) and AVT (c) scenarios; number of kernels row x ear diameter estimated in ASO (d), AVP (e) and AVT (f) scenarios and cob diameter/ear diameter ratio x ear length, estimated in ASO (g), AVP (h) and AVT (i) scenarios.

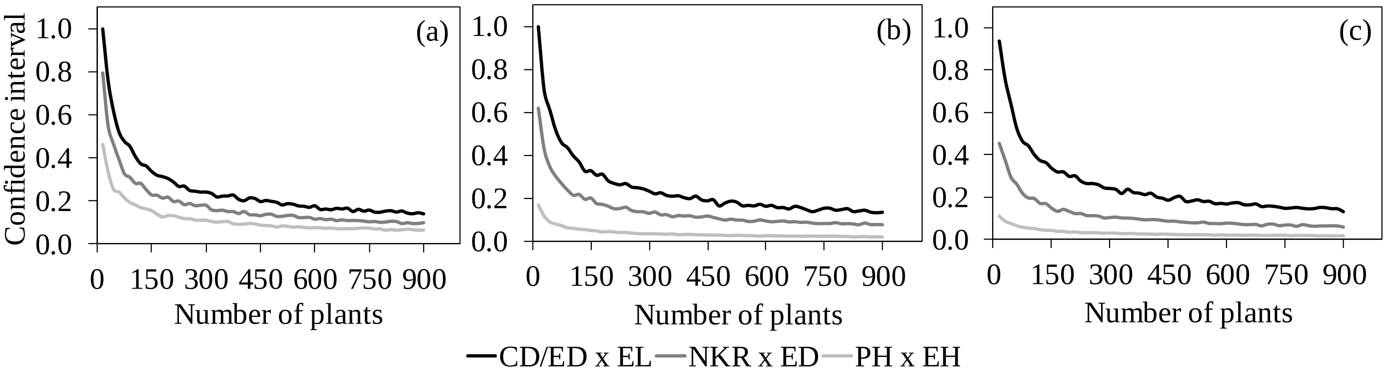

Figure 2. Amplitude of the correlation coefficient for the confidence interval of 95%. (a) ASO scenario. (b) AVP scenario and (c) AVT scenario. Lines in grey scale represent the pair cob diameter / ear diameter ratio x ear length (CD/ED x EL), number of kernels rows x ear diameter (NKR x ED) and plant height x ear height (PH x EH).

for each pair of traits were performed with the average of 60 sample sizes. Among the 165 comparisons (55 trait pairs in three scenario combinations), 164 differed. Only one did not differ. In approximately 82% of the cases, average values (AVT and AVT scenarios) overestimated the magnitude of the *r* (Table 1).

Comparing the estimated *r* in ASO × AVT scenarios, of 55 tested pairs, ten (18%) had a higher average when all sampled observations were used (Table 1). Comparing ASO × AVP scenarios, only seven combinations (13%) had a higher average *r* in correlation analysis estimated with all observations. Comparing the averages (AVP × AVT), 12 combinations (22%) were higher when the average of the plots was used (Table 1).

A *t*-test comparing the average *r* of 55 trait pairs in ASO x AVT scenario combination confirmed the difference between these (*t*-value = -12.89, *P* < 0.001). The average *r* with low magnitudes is due to the use of all pairs of correlation, where there are positive and negative values. The estimates in the ASO scenario showed a distribution similar to normal. That is related to the low asymmetry value (0.009), smaller *r* amplitude (-0.273 to 0.912), and the median value (0.268) that is similar to the average value (0.282), although the tests reject the hypothesis of normality (Kolmogorov-Smirnov = 0.048, *P* < 0.01) (Figure 3). The estimates carried out in the AVT scenario, however, shows a negative asymmetrical distribution of *r* values (-0.843), with a greater *r* amplitude (-0.552 to 0.956) and the median value (0.484), higher than the average (0.379). The distribution of *r* values in this scenario do not follow the normal distribution (Kolmogorov-Smirnov = 0.137, *P* = < 0.01) (Figure 3).

The comparison of ASO × AVP scenarios shows a behavior similar to that discussed previously, though with a slightly smaller difference (*t*-value = -9.60, *P* < 0.0001). For the AVP scenario, *r* also presented negative asymmetry (-0.566). The amplitude was also lower (-0.427 to 0.926), with a median value (0.399) higher than the average (0.350) (Figure 4). The distribution in this scenario was not normal (Kolmogorov-Smirnov = 0.136, *P* < 0.01).

The *t*-test comparing the average *r* between the AVP × AVT scenarios combinations, revealed difference (*t*-value = -3.73, *P* < 0.001). With the measures of central tendency and amplitudes of these scenarios discussed above, both showed a non-normal distribution of *r*, with a clear tendency of most of the observed values being higher than *r* average (Figure 5).

The *r* was increased by approximately 24 and 34% in the AVP and AVT scenarios, respectively. In addition, the *r* amplitude and standard deviation were higher in these scenarios (Figure 6).

Multicollinearity

Multicollinearity was considered severe for the three scenarios, regardless of the number of assessed plants (Figure 7). The use of averages (AVP and AVT scenarios) increased the CN of the correlation matrices. The largest changes occurred when the number of plants was low (n < 100). For example, with 45 and 60 plants, the CN increased by 118 and 75% for the AVP scenarios and 250 and 68% for the AVT scenario, respectively. Although in some cases the CN was higher for the ASO scenario, on mean, CN was increased by 24 and 131% in AVP and AVT scenarios, respectively (Figure 7).

DISCUSSION

The reduction of individual variation (standard deviation) observed in the scenarios AVP and AVT was the main factor responsible for overvaluing the *r* of trait pairs. This fact can be explaining due standard deviation be the divisor on correlation's formula. If covariance *XY* (dividend of formula) is similar in both scenarios, however, the standard deviation of *X* and *Y* traits (divisor of formula) are smallest, the magnitude of correlation coefficients will be greater.

The higher number of plants required for estimation of the r at the 95% CI ≤ 0.10 in trait pairs with less intensity of linear association, shows that the researcher must take into consideration the magnitude of the trait pairs, being that the confidence interval will be inversely proportional to the magnitude of its correlations. The magnitude of the CI used here (95% CI ≤ 0.10) it is not a rule, being that each researcher must adopt the appropriate confidence level for its inferences. If we consider the CI 95% CI < 0.30, 210 plants are enough for estimating trait pairs with low magnitude (r < 0.10). This number of plants it is perfectly possible of to be evaluated. The experimental design (number of treatments and repetitions) will set then, the number of plants to be sampled in each plot. In experiments with large numbers of experimental units (e.g. factorial designs), the increase in sample size will provide greater confidence in the estimates provided that they are properly followed the sampling procedures and maintained traceability of these samples.

Although for trait pairs with high linear association (EH x PH) AVP and AVT scenarios needed 83 and 89% fewer plants to estimate r, the average *r* in these scenarios was increased by 11 and 15%, respectively, compared to the ASO scenario (*r* = 0.83). In an analysis that depends on of the linear correlation matrix for their estimates, e.g., canonical correlation, path analysis and stepwise multiple linear regression procedures, high linear association magnitudes among explanatory traits make it difficult to analyze, threatening the statistic and the inferential interpretation (Graham, 2003).

A recent study revealed that multicollinearity begins to seriously distort the estimates of the path coefficients when the explanatory traits show *r* > | 0.7 | (Dormann et al., 2013). While there have been observed high

Table 1. *t*-statistics for the average correlation coefficient (*r*) of 55 trait pairs estimated in 60 different numbers of plants.

Trait pairs	ASO x AVT			ASO x AVP			AVP x AVT		
	Average r		*t*	Average r		*t*	Average r		*t*
	ASO	AVT		ASO	AVP		AVP	AVT	
EH x PH	0.834	0.955	782.08**	0.834	0.955	-782.08**	0.925	0.955	484.84**
EL x PH	0.249	0.573	944.63**	0.249	0.414	-460.71**	0.414	0.573	547.30**
EL x EH	0.215	0.546	1079.40**	0.215	0.399	-559.79**	0.399	0.546	516.39**
ED x PH	0.478	0.750	910.05**	0.478	0.641	-559.57**	0.641	0.750	389.69**
ED x EH	0.458	0.712	805.67**	0.458	0.610	-558.02**	0.610	0.712	315.06**
ED x EL	0.417	0.513	205.64**	0.417	0.514	-113.86**	0.514	0.513	-1.62ns
NRE x PH	0.234	0.447	458.76**	0.234	0.360	-241.01**	0.360	0.447	155.68**
NRE x EH	0.160	0.346	550.12**	0.160	0.282	-290.51**	0.282	0.346	154.48**
NRE x EL	0.028	0.040	38.31**	0.028	0.082	-107.56**	**0.082**	**0.040**	-93.21**
NRE x ED	0.498	0.621	391.20**	0.498	0.578	-248.07**	0.578	0.621	224.23**
NKR x PH	0.234	0.568	1008.10**	0.234	0.402	-511.47**	0.402	0.568	534.93**
NKR x EH	0.206	0.519	942.17**	0.206	0.387	-482.23**	0.387	0.519	379.25**
NKR x EL	**0.646**	**0.618**	68.570**	0.646	0.659	-26.22**	0.659	0.618	-103.53**
NKR x ED	0.319	0.334	22.55**	0.319	0.394	-83.45**	**0.394**	**0.334**	-73.38**
NKR x NRE	0.067	0.092	58.63**	0.067	0.164	-124.62**	**0.164**	**0.092**	-95.29**
CD x PH	0.256	0.416	253.86**	0.256	0.376	-246.66**	0.376	0.416	59.46**
CD x EH	0.313	0.488	248.73**	0.313	0.439	-215.07**	0.439	0.488	66.05**
CD x EL	0.308	0.359	98.98**	0.308	0.351	-76.61**	0.351	0.359	14.34**
CD x ED	0.653	0.730	263.58**	0.653	0.729	-325.70**	0.729	0.730	2.64*
CD x NRE	**0.269**	**0.259**	23.550**	0.269	0.298	-59.45**	0.298	0.259	-69.79**
CD x NKR	0.069	0.086	294.860**	**0.069**	**0.064**	7.30**	0.064	0.087	-228.23**
CL x PH	0.222	0.485	555.35**	0.222	0.369	-339.33**	0.369	0.485	288.34**
CL x EH	0.170	0.449	552.07**	0.170	0.340	-388.77**	0.340	0.449	225.58**
CL x EL	0.908	0.936	171.82**	0.908	0.923	-69.04**	0.923	0.936	70.45**
CL x ED	0.430	0.479	79.17**	0.430	0.523	-106.13**	**0.523**	**0.479**	-56.79**
CL x NRE	**0.023**	**0.003**	53.69**	0.023	0.065	-82.83**	**0.065**	**0.003**	-152.69**
CL x NKR	**0.639**	**0.592**	136.66**	0.639	0.647	-26.52**	**0.647**	**0.592**	-160.26**
CL x CD	0.343	0.393	74.94**	0.343	0.391	-69.94**	0.391	0.393	3.76**
TNK x PH	0.303	0.642	998.75**	0.303	0.488	-556.69**	0.488	0.642	471.33**
TNK x EH	0.226	0.556	1051.70**	0.226	0.419	-564.68**	0.419	0.556	399.82**
TNK x EL	**0.548**	**0.493**	147.44**	**0.548**	**0.540**	11.17**	**0.540**	**0.493**	76.05**
TNK x ED	0.532	0.639	192.04**	0.532	0.594	-110.93**	0.594	0.639	70.25**
TNK x NRE	0.519	0.691	350.18**	0.519	0.625	-242.68**	0.625	0.691	121.83**
TNK x NKR	0.719	0.736	69.52**	0.719	0.777	-136.53**	**0.777**	**0.736**	110.79**
TNK x CD	**0.191**	**0.116**	180.57**	**0.191**	**0.179**	31.27**	**0.179**	**0.116**	144.95**
TNK x CL	**0.535**	**0.428**	274.75**	**0.535**	**0.502**	56.70**	**0.502**	**0.428**	148.45**
CD/ED x PH	-0.123	0.2273	235.70**	-0.123	-0.174	120.65**	-0.174	0.227	111.10**
CD/ED x EH	-0.034	0.0840	91.19**	-0.034	-0.051	33.37**	-0.051	0.084	65.52**
CD/ED x EL	0.002	0.0400	84.15**	0.002	-0.079	124.89**	-0.079	0.040	55.65**
CD/ED x ED	**-0.121**	**0.0576**	88.49**	**-0.121**	**-0.078**	-57.34**	**-0.078**	**0.058**	29.73**
CD/ED x NRE	-0.13	0.3127	210.45**	-0.130	-0.219	116.49**	-0.219	0.313	86.75**
CD/ED x NKR	-0.221	0.4987	717.16**	-0.221	-0.360	355.08**	-0.360	0.499	336.64**
CD/ED x CD	**0.666**	**0.636**	80.68**	**0.666**	**0.620**	120.02**	0.620	0.636	62.00**
CD/ED x CL	0.038	0.048	20.31**	0.038	-0.029	126.76**	-0.029	0.048	146.04**
CD/ED x TNK	-0.265	0.5475	504.87**	-0.265	-0.421	301.55**	0.421	0.547	190.86**
TKW x PH	0.405	0.638	505.66**	0.405	0.539	-329.62**	0.539	0.638	254.21**
TKW x EH	0.418	0.674	537.91**	0.418	0.553	-335.17**	0.553	0.674	286.18**
TKW x EL	0.364	0.594	591.29**	0.364	0.452	-214.08**	0.452	0.594	432.89**
TKW x ED	0.488	0.685	617.07**	0.488	0.623	-499.34**	0.623	0.685	214.27**

Table 1. Contd

TKW x NRE	**-0.206**	**0.0141**	626.90**	**-0.206**	**-0.082**	-223.54**	**-0.082**	**0.014**	130.71**
TKW x NKR	0.096	0.209	288.70**	0.096	0.140	-104.59**	0.140	0.209	159.98**
TKW x CD	0.482	0.738	564.25**	0.482	0.644	-472.08**	0.644	0.738	204.82**
TKW x CL	0.384	0.550	305.53**	0.384	0.471	-182.82**	0.471	0.550	164.41**
TKW x TNK	-0.102	0.130	528.63**	-0.102	0.013	-229.57**	0.013	0.130	277.06**
TKW x CD/ED	0.163	0.314	338.57**	0.163	0.236	-197.08**	0.236	0.314	172.82**

Average values represent 1000 bootstrap simulations of the original data coming from all sampled observations (ASO), coming from the average of each plot (AVP) and coming from the average of treatments (AVT). Coefficients in bold indicate the pairs in which *r* was lower with the use of averages. '*' and '**' show the significances at 0.001 and 0.01 of probability level, respectively. 'ns' is not significant. PH, Plant heigth; EH, ear heigth; EL, ear length; ED, ear diameter; NRE, number of rows per ear; NKR, number of kernels per row; CL, cob length; CD, cob diameter; CD/ED, cob diameter / ear diameter ratio; TNK, total number of kernels per ear; TKW, thousand-kernel weight.

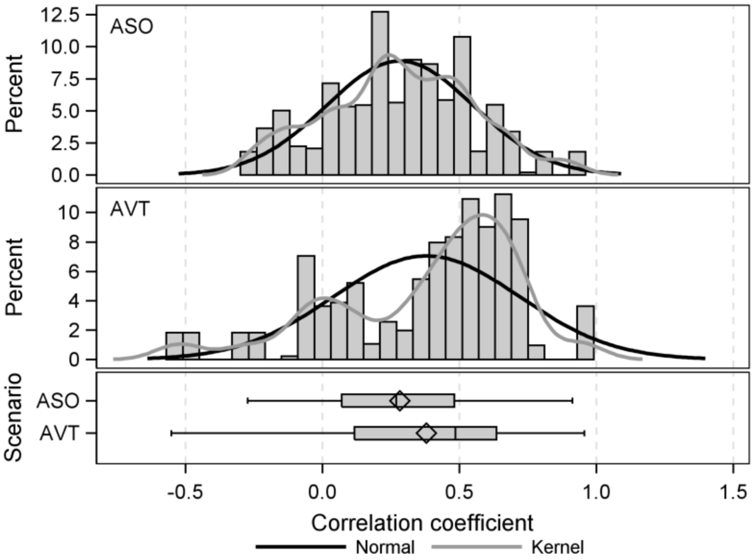

Figure 3. Distribution of average values of correlation coefficient in ASO x AVT scenarios combination. Columns represent the observed values. Black and gray lines represent the normal distribution and Kernel density estimation, respectively. ASO and AVT scenarios represent the correlation coefficients estimated by all sampled observations, and by the average values of treatments, respectively. In the lower plot, the average (rhombus), the median (vertical line), the distance between the 25th and 75th percentiles (length of the box) and the maximum and minimum values (outer spread) of the estimated correlation coefficient are presented for each scenario.

correlations in the ASO scenario (e.g. EH x PH, $r = 0.83$), the higher values for the same pair ($r = 0.92$) and ($r = 0.96$) estimated in APV and AVT scenarios, respectively, demonstrated that these data arrangement methodologies overestimate the magnitude of the *r* and may result in larger problems in estimates of multiple regression parameters, leading to an erroneous interpretation of predictors in a statistical model. Thus, these methods must be carefully evaluated by the researchers when the goal is to use the correlation matrix in studies involving multiple regression, as for this, the independence or the less degree of dependence among explanatory traits is sought (Prunier et al., 2014; Montgomery et al., 2012)

Average values (AVP and AVT scenarios), visibly elevated the multicollinearity of the matrices, confirming the earlier discussion. Although there are variations in CN in each studied sample size, the multicollinearity was increased on average by 24 and 131% when the AVP and AVT scenarios were considered in the estimation of correlation matrices. Although there are techniques for adjusting the multicollinearity as to delete the traits responsible for inflating the variance of the coefficients

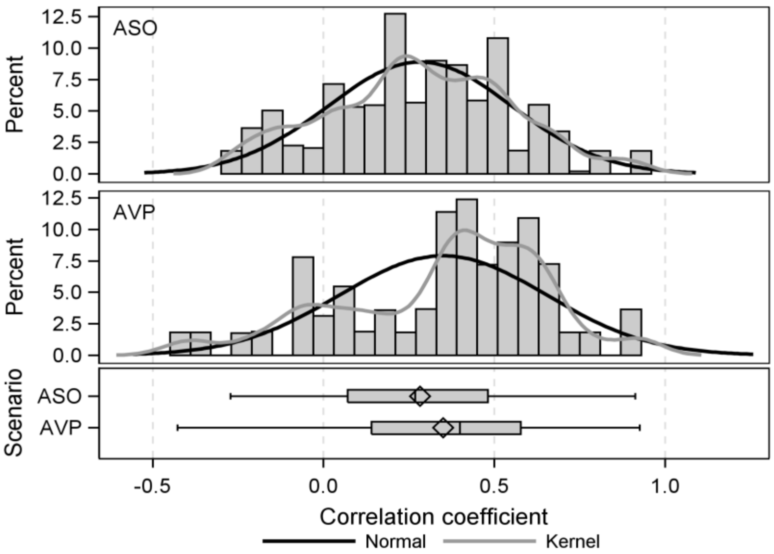

Figure 4. Distribution of average values of correlation coefficient in ASO x AVP scenarios combination. Columns represent the observed values. Black and gray lines represent the normal distribution and Kernel density estimation, respectively. ASO and AVP scenarios represent the correlation coefficients estimated by all sampled observations, and by the average values of plots, respectively. In the lower plot, the average (rhombus), the median (vertical line), the distance between the 25th and 75th percentiles (length of the box) and the maximum and minimum values (outer spread) of the estimated correlation coefficient are presented for each scenario.

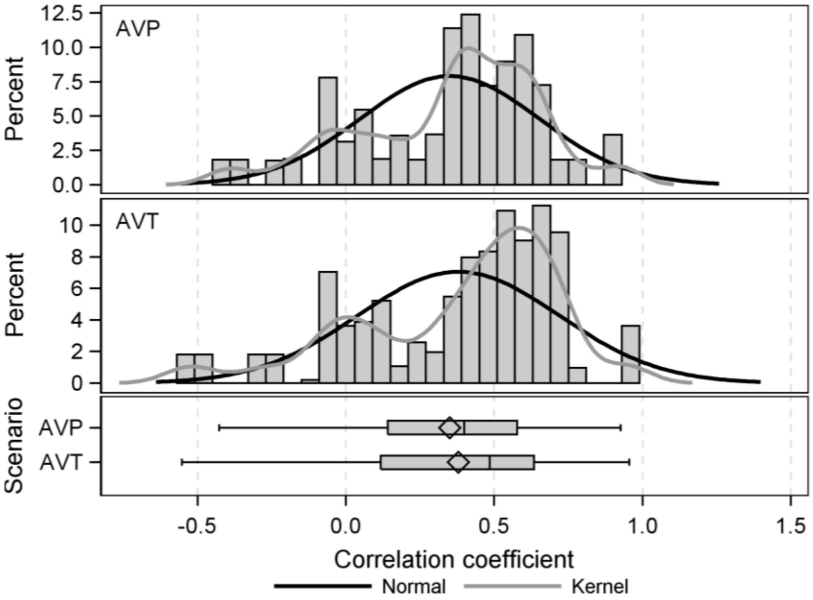

Figure 5. Distribution of average values of correlation coefficient in AVP x AVT scenarios combination. Black and gray lines represent the normal distribution and Kernel density estimation, respectively. AVP and AVT scenarios represent the correlation coefficients estimated by average values of plots and treatments, respectively. In the lower plot, the average (rhombus), the median (vertical line), the distance between the 25th and 75th percentiles (length of the box) and the maximum and minimum values (outer spread) of the estimated correlation coefficient are presented for each scenario.

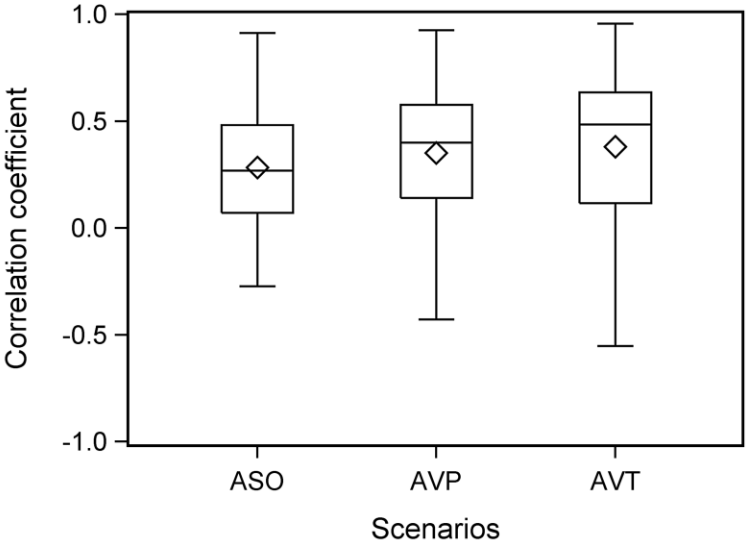

Figure 6. Descriptive analysis of correlation coefficients of 55 trait pairs estimated in 60 sample sizes by 1000 bootstrap simulations. Scenarios represent the original data coming from all sampled observations (ASO), coming from average values of each plot (AVP) and coming from average values of treatments (AVT). The rhombus within the box represents the average in the scenario. The horizontal line within the box represents the median value. The length of the box is the distance between the 25th and 75th percentiles. Outer spread represents the maximum and minimum values.

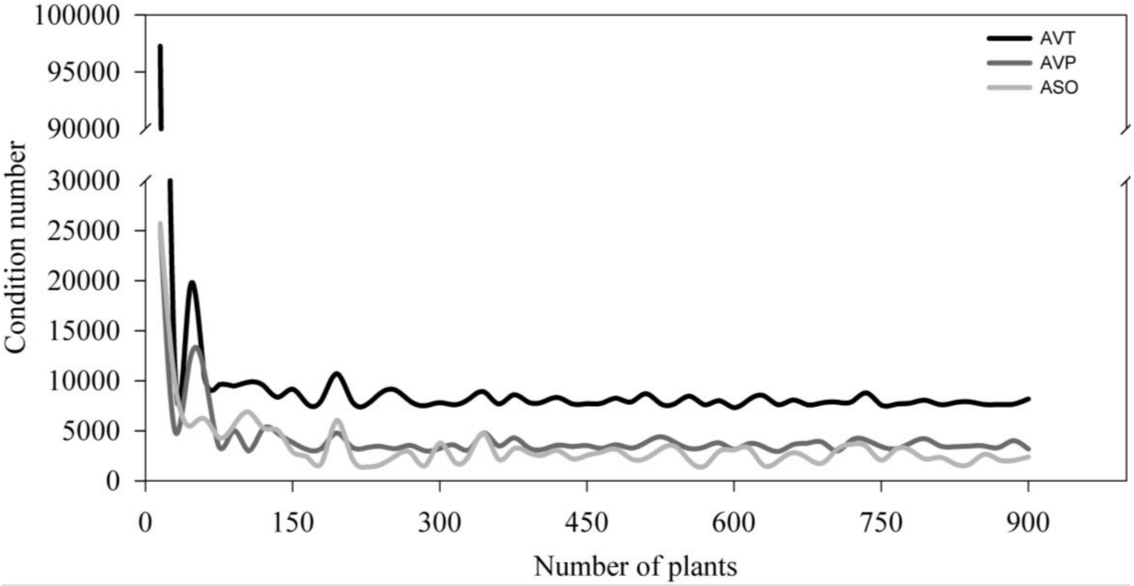

Figure 7. Condition number of correlation's matrices among explanatory traits estimated with 60 different sample sizes. For each sample size, the traits' values were estimated by average of 1000 bootstrap simulations of the original data coming from all sampled observations (ASO), coming from average values each plot (AVP) and coming from average values of treatments (AVT).

(Gunst and Mason, 1977) or to perform estimates by using equations partially modified by the inclusion of a *k* constant in the diagonal elements of correlation matrix (Hoerl and Kennard, 1970a), these techniques can mask the true biological behavior's response, because the deletion of the traits can reduce the model's explanation power. The inclusion of the k constant is effective in reducing the magnitude of multicollinearity, however, also causes a bias in the regression analysis (Hoerl and Kennard, 1970a)

The best strategy to mitigate the problems caused by multicollinearity is to reduce it since it becomes practically impossible to eliminate it. In this research, a simple method for mitigating the multicollinearity in correlation matrices is suggested: estimating the correlation coefficients considering all observations, maintaining traceability and individual variance of the sample. This can be accomplished without significant increase of time, labor and financial resources since, a priori, all sampled plants were assessed.

Conclusion

Estimates made with data based on averages (AVP and AVT scenarios) reduce the individual variances, overestimate the correlation coefficients and increase the multicollinearity in correlation matrices. Thus, studies that require explanatory traits in order to predict a dependent trait will present greater misstatements in the estimates of the regression coefficients, if these methods are used. By using values coming from all sampled plants, 210 plants are enough for estimating Pearson product-moment correlation coefficients among maize traits in the bootstrap confidence interval of 95% < 0.30. The current study about data arrangement on Pearson's correlation coefficients presents useful information on the planning of future experiments in plant breeding involving biometric templates that require the correlation matrix for their estimates.

Conflict of Interests

The authors have not declared any conflict of interests.

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Abbreviations

ASO, all sampled observations; AVP, average values of plot; **AVT**, average values of treatments; **CD**, cob diameter; **CD/ED**, **cob** diameter/ear diameter ratio; **CL**, cob length; **ED**, ear diameter; EH, ear height; **EL**, ear length; **NKR**, number of kernels per row; **NRE**, number of rows per ear; **PH**, plant height; **TKW**, thousand-kernel weight; **TNK**, total number of kernels per ear.

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Mineral and organic fertilizer in two *Physalis* species

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***Physalis* cultivation can be an alternative of extra income for small and medium producers, mainly with the use of materials available on the property that can replace chemical fertilizer. This study aimed to evaluate agronomic parameters of *Physalis peruviana* and *P. pubescens* submitted two different sources of fertilization - chemical and organic. We evaluated plant height, shoot dry mass, production/plant, fruits diameter and weight and productivity. The organic fertilization provided better results for vegetative parameters. Regarding production, fertilization resulted in increase in mass, with no significant difference between sources. In the production of fruits/plant we obtained 156.2 g, 274.6 and 355.5 g for unfertilized, chemical and organic fertilizers, respectively, without significant differences between species. The productivity estimates were 2,370, 1,831 and 1,041 kg ha^{-1}, for organic, mineral and unfertilized treatments, respectively. These results demonstrate that organic fertilizer with poultry manure is the best alternative as a source of nutrients, which may result in gains to the producer by the use of originated waste from other activities, as well as lower environmental contamination, either by improper disposal of waste or the use of chemical fertilizers.**

Key words: Nitrogen, nutrition, poultry litter, production, Solanaceae

INTRODUCTION

The *Physalis* genus, belonging to the Solanaceae family, comprises about 120 species and its probable center of origin is the Mexico (Li et al., 2008; Kindscher et al., 2012; Whitson, 2012). Its fruits are protected by a capsule and contain significant levels of vitamins A, C and B complex, and minerals, carotenoids and tocopherols (Hassanien, 2011; Ramadan, 2011), with some of these with antioxidant activity (Bravo et al., 2015). It is noteworthy the production of polioxigenated metabolites, the vitasteroids, including physalins, which are substances that present interesting pharmacological activities (Tomassini et al., 2000). Some species are used in traditional medicine for treatment of diseases, with several studies demonstrating the presence of other bioactive molecules that can cause future, medications (Arun and Asha, 2007; Chen et al., 2011; Hassanien, 2011; Puente et al., 2011).

Physalis, although little known to the majority of consumers, has production and marketing potential, especially for its nutraceutical qualities (Barbieri et al., 2012), and may be an excellent alternative for small and medium producers (Thomé and Osaki, 2010). In Brazil,

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Table 1. Area soil testing results where the experiment was carried out.

MO ($g\ dm^{-3}$)	P ($mg\ dm^{-3}$)	K ($cmol_c\ dm^{-3}$)	Ca ($cmol_c\ dm^{-3}$)	Mg ($cmol_c\ dm^{-3}$)	pH $CaCl_2$	V (%)	Index SMP	SB ($cmol_c\ dm^{-3}$)	H+Al ($cmol_c\ dm^{-3}$)
34.85	23.44	0.70	4.53	1.78	4.60	58.56	6.00	7.01	4.96

Table 2. Results of the analysis of organic matter (poultry litter after weathering).

N (%)	P (%)	K (%)	Ca (%)	Mg (%)
2.65	1.50	2.29	2.25	0.51

Table 3. Amount of each fertilizer applied per plant.

Fertilizer	g $plant^{-1}$
Urea	95.0
Single superphosphate	311.1
Potassium chloride (KCl)	74.0
Poultry litter	1600.0

P. peruviana has been gaining attention among producers; however, are still scarce work and research results to guide them in relation to cultural practices, productivity and economic aspects of production. Currently, the fertilizer management carried out in Phyalis crops is based on recommendations for tomato crop (Gonçalves et al., 2012; Filgueira, 2003; Rufato et al., 2008). Differently from *P. peruviana, P. pubescens* is known by farmers to be considered weed (Lorenzi, 2008), and, therefore without major interest in the exploitation of its fruits.

The application of fertilizers and lime in the fruit production can represent over 25% of the production cost and, therefore, the use of organic compounds becomes a promising alternative (Vidigal et al., 2010). In addition, the use of organic fertilizer provides greater environmental sustainability, preserving natural resources, avoiding water contamination and harnessing materials available on the agricultural property, reducing production costs and making it feasible economically.

Based on the presented, the aim of this work was to evaluate agronomic parameters of two species of *Physalis* - *P. peruviana* L. and *P. pubescens* L. - submitted to mineral and organic fertilizer (poultry litter), with focus on nitrogen fertilization.

MATERIALS AND METHODS

Seeds of *P. peruviana* L. and *P. pubescens* L. were obtained from plants grown in a greenhouse. It was used 250 mL plastic cups with fertile humus as substrate to obtain the seedlings, setting three seeds in each one. The cultures were kept in a greenhouse, with average temperatures in the period of 24 °C, being carried manual waterings daily, in the early morning and late afternoon, avoiding waterlogging and/or water stress. After 20 days, was performed the thinning leaving one seedling cup. When the plants reached about 15 cm (50 days after sowing) were transplanted to the experiment site in spacing of 3,0 x 0,5 m. Each experimental unit consisted of 12 plants, and the borders were not used for evaluations. The crop treatment consisted of frequent waterings (manual) with soil kept in its field capacity, weed control (manual weeding), especially in the early stages and monitoring the emergence of pests and diseases. Once the plants reached 30 cm were vertically trained with the help of bamboo stakes.

The experiment was conducted within the municipal district of Itapejara D'Oeste/PR, located at 26°3'30.54" S and 52°48'7.06" O, altitude of 530 m, with the weather classified as Cfa (humid subtropical climate), according to Köppen. The average temperature during the execution of the experiment was 23.5°C (Data collected daily at the site of the experiment, with thermometer use. The soil was analyzed before the experiment installation (Table 1) and after correction, presented pH 5.8 and base saturation (V%) above 70%.

The two species of *Phyalis* received, each one, three treatments: control (without fertilizer), mineral fertilizer (NPK) and organic (poultry litter). For mineral fertilizers was used three different sources: urea (45% N), single superphosphate (18% P_2O_5) and potassium chloride (60% K_2O). The organic fertilization consisted of existing poultry litter on the property where the experiment was carried. This material was weathered for a period of 90 days before use, was submitted after it, to chemical analysis to determine nutrient content, as shown in Table 2.

Fertilization was performed in the pits at the time of transplanting, considering 300 kg of nitrogen per hectare. The nitrogen dose was based on trials in greenhouse (Passos, 2013). The amounts of phosphorus and potassium were calculated on the basis of the result of poultry litter analysis. The doses used are shown in Table 3.

The experimental design was a randomized blocks, with two species, three treatments and five blocks, characterized as a two-factor (2 x 3) with two qualitative variables, with 30 plots and each one with 12 plants.

The experiment was carried in the years 2013 and 2014 (from October 2013 to January 2014 having as previous experiment based on 2012 to 2013), and due to irregularity of production, was set up a unique collection of materials at 140 days after sowing. The following parameters were evaluated: plant height (cm), dry matter of aerial parts (DMAP) (g), fruit production per plant (g), average yield (kg ha^{-1}), transverse diameter (equatorial) average fruit (mm) and average fruit weight, with capsules (g). It was also estimated the relative increase in productivity arising nitrogen fertilization (IRPAN) proposed by Ferreira et al. (2010). For this, we used the difference between the maximum production fruit (MW) kg ha^{-1} and fruit production to a zero dose (PBC zero) divided by N rate required to obtain the PM by using the formula: IRPAN = (PM – PBC zero) / (Dose PM).

The data were submitted to analysis of variance (ANOVA) and the means analyzed by Tukey test at 5% probability of error, with GENES statistical program.

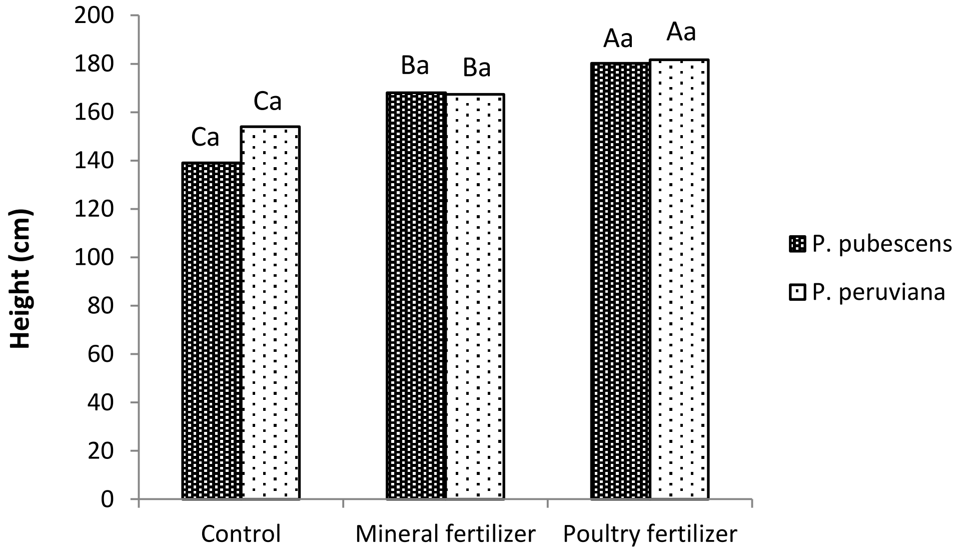

Figure 1. Final plant height (cm) of *Physalis*, under different nutritional

RESULTS AND DISCUSSION

The final plant height parameter showed no significant difference between species, however, there was significant difference between the nutritional sources. Fertilization providing higher height was organic, with an average of 180.90 cm main branch height, against 167.7 of mineral fertilizer (Figure 1).

Peixoto et al. (2010), using two doses of cattle manure, 30 and 60 t ha^{-1}, *Physalis (P. peruviana),* found no significant difference between them in plant height parameter, showing that although there is no influence of dose, probably the source was important. In tomato plants, the application of the effluent derived from the biodigestion of cattle manure resulted in higher plants (10.5%) compared to treatment with NPK (Campos, 2007). Freitas et al. (2012) also observed a better response in the rate of growth of sorghum plants when received organic manure (cattle manure). These authors suggest that plants under the organic fertilizer respond gradually over the growing cycle of the crop, once the nutrient release rate is not as fast as in the chemical fertilizer, supplying therefore their nutritional needs for a longer period chemicals, which could be observed in the same Physalis plants fertilized with poultry litter.

Unlike that observed for *Physalis*, some studies comparing organic and chemical fertilizers, such as jambu, the authors had greater heights when plants were treated with urea, and organic resulted in lower plants than the considered for the specie (Borges et al., 2013).

The dry matter of aerial parts (DMAP) showed the same pattern of plant height parameter. The organic fertilization resulted in better response, with an average of 1010.35 g of DMAP plant^{-1}, the mineral fertilizer 982.96 g DMAP plant^{-1}, and the control with the lowest value of 882.10 g DMAP plant^{-1} (Figure 2).

Satisfactory results resulting from organic fertilization were found also in other species, such as basil, which in addition to chicken manure, received mineral complementation (Blank et al., 2005). Silveira Junior et al. (2015) reported an increase in Piata grass biomass using different doses of biofertilizers derived from the poultry manure digestion. Beyond merely supplying nutrients, the positive effects in the soil such as increased microbial activity, improved aeration and water infiltration in the soil profile, it provided better plant growth, resulting in the largest mass production with organic fertilizer, in the two species tested, in relation to controls and mineral fertilizer. The opposite was also observed in study about the influence of semi-decomposed poultry manure (10 t ha^{-1}) and mineral fertilizers (4.5 g m^{-2}), applied in coverage, in lettuce and peruvian carrot, in isolated crops or in consortium (Vieira et al., 2003). The authors obtained higher yields of fresh and dry weight of lettuce in consortium with Peruvian carrot and mineral N (9.88 t ha^{-1} fresh weight and 0.53 t ha^{-1} dry mass). With organic fertilizer under the same conditions (consortium), was obtained 6.68 t ha^{-1} and 0.39 t ha^{-1}, respectively. In this case, it appears that the immediate availability of N, as well as intercropping favored in some way, the target species. Also in lettuce, Steiner et al. (2012) achieved a higher dry matter when applied poultry litter, however, did not differ from pig slurry and chemical treatment with urea. Borges et al. (2013), working with jambu, a plant widely used in northern Brazil, found greater accumulation of biomass in chemical fertilizer in comparison to cattle manure, however, the latter promoted greater phosphorus accumulation in the leaves.

Regarding the size (diameter) of the fruits, without the capsules, there was no significant difference for the

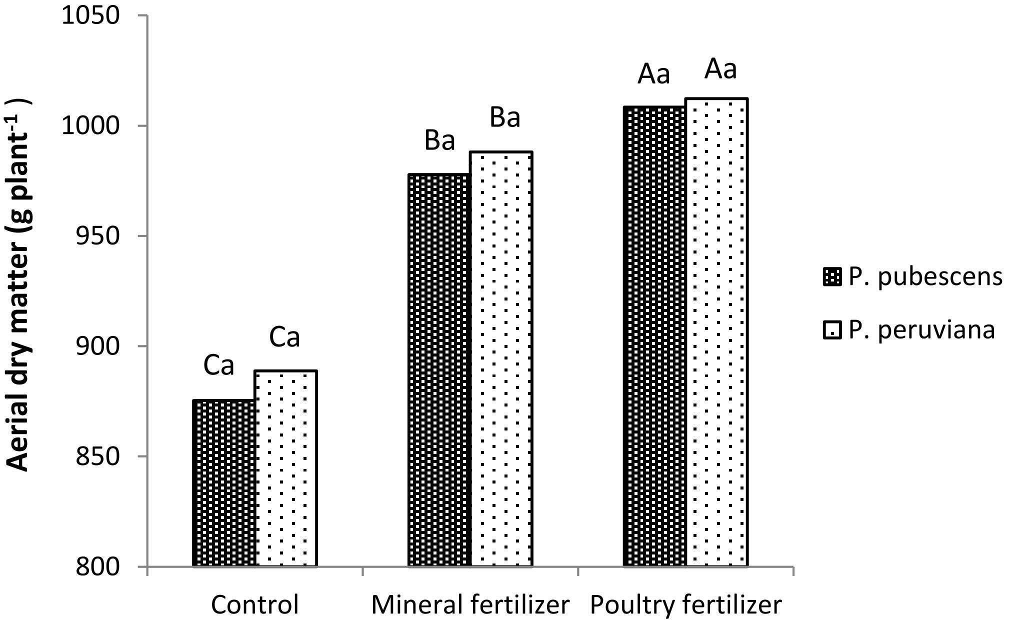

Figure 2. Dry matter of aerial parts (g plant^{-1}) *Physalis*, under different nutritional sources. Uppercase compare the different nutritional and lowercase letters, the two species, by Tukey test (5%).

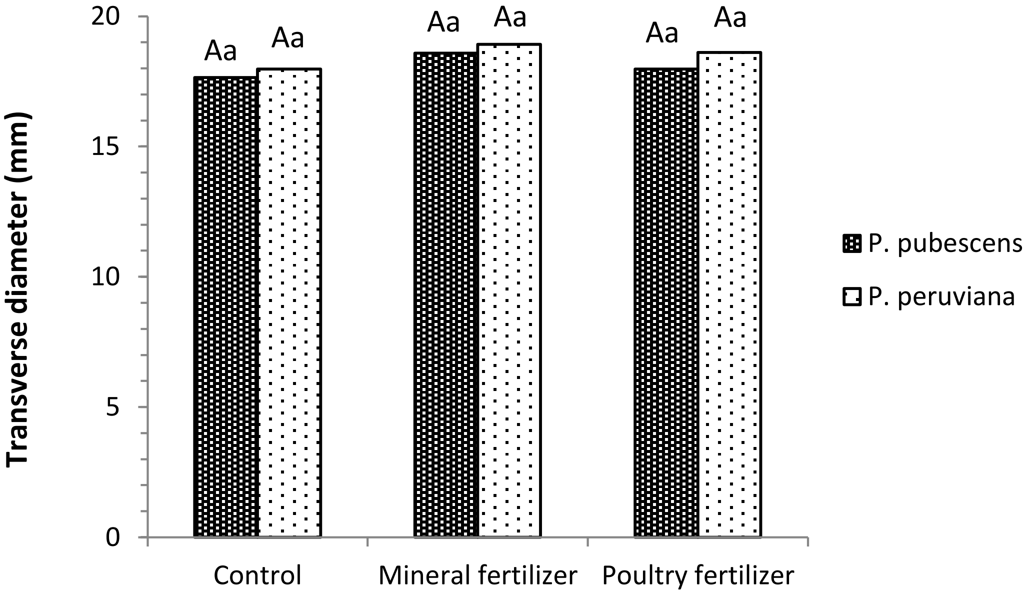

Figure 3. Average fruit diameter (mm) of *Physalis*, under different nutritional sources. Uppercase compare the different nutritional and lowercase letters, the two species, by Tukey test (5%).

different species and for the tested nutritional sources (Figure 3). The average values for both species were 17.81 mm (control), 18.75 mm (chemical fertilizer) and 18.30 mm (organic).

These results differ from those observed by Muniz et al. (2011), that when evaluating the effect of chemical fertilizer (NPK 5:20:10) and organic (manure compost of bovine 50% + 50% swine) in *P. peruviana* obtained larger diameter fruit in plants fertilized with the organic compound (21.14 mm with coat); and the chemical resulted in fruits with a mean diameter of 19.98 mm and 18.99 mm control. Albayrak et al. (2014), analyzing only

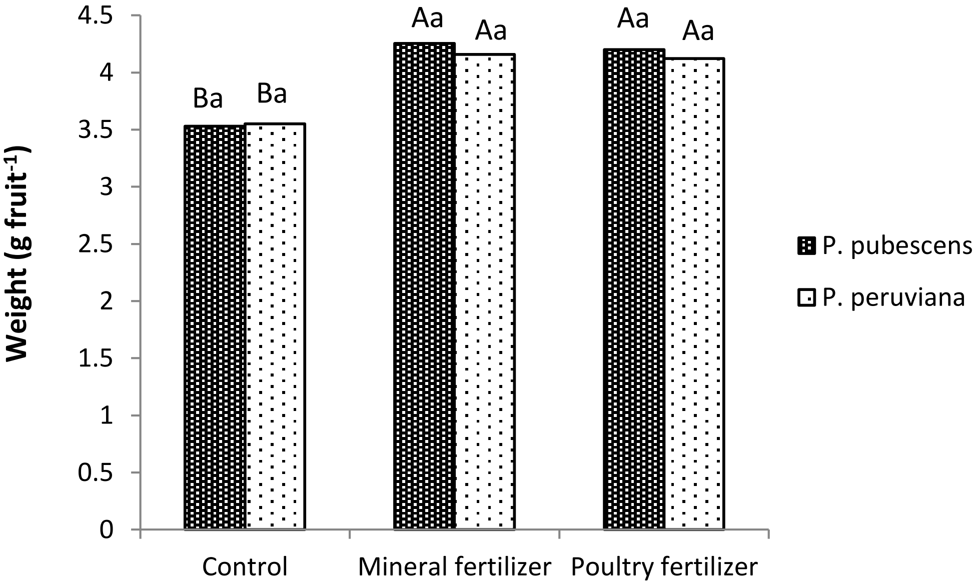

Figure 4. Fruit average mass (g), with capsule, of *Physalis*, under different nutritional sources. Uppercase compare the different nutritional and lowercase letters, the two species, by Tukey test (5%).

the influence of nitrogen, obtained no difference in fruit diameter of *P. peruviana* with increasing doses of this element (from 0.4 to 2 kg ha^{-1}), considering that the control resulted in significantly smaller fruits.

Colombian standards classify the fruits of "uchuva" (*P. peruviana*) in four marketing categories, with relative to the equatorial diameter, with coat: A - 15.0 to 18.0 mm; B - 18.1 to 20.0 mm; C - 20.1 to 22.0 mm; D 22.0 mm (Codex Stan, 2005). Therefore, the fruits of both species of *Physalis* would be within the marketing standards of the largest producer in the Americas. Considering that the fruits were measured without coats, the control with fruit classified at least in category A (17.81 mm), and B or C to the fruits from plants fertilized (18.75 and 18.30 mm, chemistry and organic, respectively). It is clear other factors can contribute to the improvement of the size of the fruit, such as spacing, conduct of the plant, etc. and may result in bigger fruit.

The average masses of fruits (with cap) were enriched with the supply of nutrients, however, there was no significant difference between organic and mineral sources. The organic fertilizer produced fruits averaging 4.2 g, 4.1 g against chemical and 3.5 g for the control (Figure 4).

These results are close to those observed by Muniz et al. (2011) in fruit of *Physalis* derived from plants treated with chemical fertilizer (4.67 g) and organic (4.43 g). Thomé and Osaki (2010) tested different levels of NPK in producing three kinds of *Physalis* (*P. peruviana, P. ixocarpa*, and *P. angulata*) and obtained average fruit mass without significant differences between species, ranging from 1.15 to 1.44 g and only *P. peruviana* and *P. ixocarpa* tended to increase in mass with the increase of fertilizer. In tomatoes produced in the greenhouse, Hernández et al. (2014) did not observe significant differences between mineral and organic fertilizer (manure of sheep + goats and mixing of waste from olive oil industry + manure + pruning of olive).

According to the export standards (Colombia), the *Physalis* fruit should be sold with a minimum weight of 4.0 g (Codex Stan, 2005). Therefore, as seen with the diameter, the fruits produced in the experiment, other than from the control, would be considered acceptable by Colombian standards.

When was analyzed production per plant parameter, there were significant differences in relation to different sources of fertilizer. Both species and the interaction between the factors were not significant. The average fruit yield plant^{-1} was 156.19 g (control), of 274.64 g (chemical fertilizer), and 355.44 g (organic), indicating that both species of *Physalis* showed higher fruit production plant^{-1} when subjected to the latter, with an increase of more than 40% compared with the control (Figure 5).

In this study, although no significant difference between species was found, it was observed that *P. peruviana* excelled when fertilized with poultry litter. In a previous study only with mineral nitrogen fertilization at a dose of 300 kg ha^{-1}, *P. peruviana* produced more fruit, but with smaller individual mass (unpublished data).

In an experiment with three species of *Physalis*, Tomé and Osaki (2010) found that increases in NPK doses did not change the production of fruits per plant, and even between species, there was no significant difference with

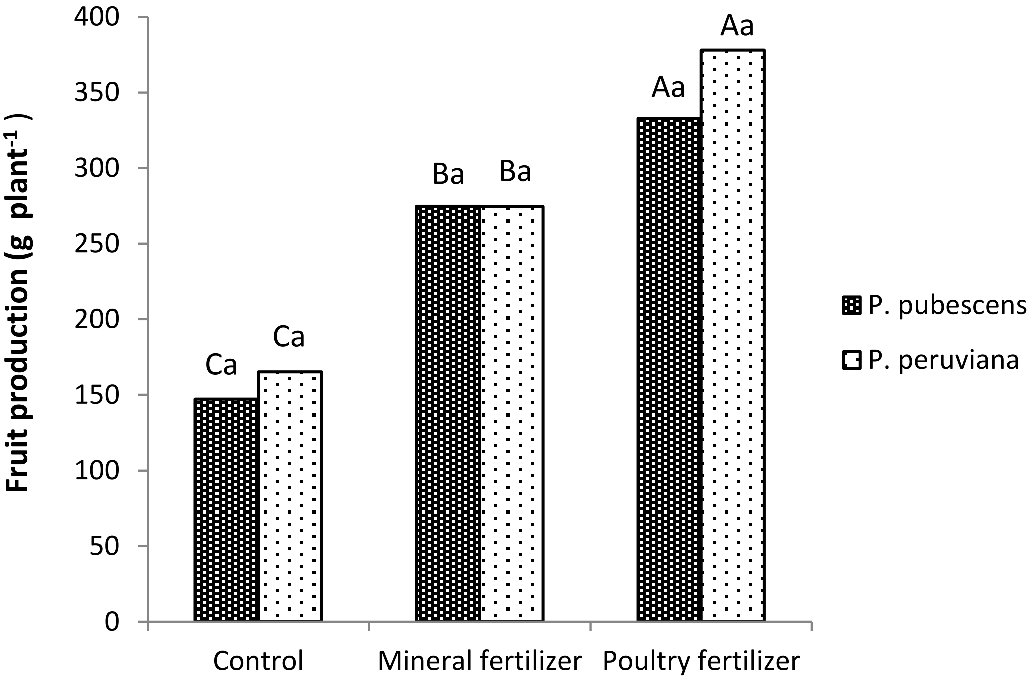

Figure 5. Production (g of fruit plant^{-1}) of *Physalis*, under different nutritional sources. Uppercase compare the different nutritional and lowercase letters, the two species, by Tukey test (5%).

the values being between 1.19 to 1.20 kg of fruit plant^{-1}. Having been set a single harvest, at 140 days after sowing, fruit production in this study was much lower, however, with the continued fruitfulness would be possible to reach higher values, even higher than the authors mentioned above.

Hérnandez et al. (2014), when analyzing the effect of chemical fertilization and two forms of organic compounds (manure of sheep + goats and mixing of waste from for olive oil industry + manure + pruning olive) in tomato production in a greenhouse, they found that the latter were those that produced few fruit by plants - on average 19 and 7.75, respectively, while the control (chemical fertilizer) produced 73.5 fruits per plant. In cucumber, the effect of organic fertilizer of poultry manure base (4 t ha^{-1}) was superior to chemical treatment (NPK), producing more fruit, of better quality and size (Okoli and Nweke, 2015).

With regard to productivity, due to the irregular fruiting, lasting several weeks, approximations were made, taking into account four months of production. The average yields were obtained from 1,041 kg ha^{-1} for the control, 1,831 kg ha^{-1} for chemical fertilizer and 2,370 kg ha^{-1} for organic. Em tomato plants, Muller et al. (2013) found lower productivity of commercial fruits with poultry litter (16.2 t ha^{-1}), reaching 86.9 t ha^{-1} against 100.1 t ha^{-1} with chemical complementation (500: 600: 600 kg ha^{-1} t NPK), in the 2007/2008 harvest, suggesting that the low result of organic fertilizer was due to an imbalance of nutrients or lack of simultaneity between the mineralization of nutrients and plant needs. The same was observed by Hernandez et al. (2014), with tomatoes produced in the greenhouse, with the chemical fertilization overcoming organic in relation to production. Tomato, grown in poor soil (andosol) and imbalance between cations, showed no significant difference in the number of fruits per plant and productivity, for the treatments with chemicals, organic manure (poultry manure) or a combination of both (Tonfack et al., 2008). Araújo et al. (1999) had lower productivity of melon with organic fertilizer when compared to mineral. Since Rech et al. (2006) found that increased doses of organic fertilizer (poultry litter) were able to increase the number of fruit per plant zucchini, but not significantly differ from the mineral fertilizer.

When the gains are estimated with fertilization, through IRPAN, it was found that the organic source, regardless of the species resulted in increased 4.43 kg ha^{-1} of fruits while the chemical was only 2.63 kg ha^{-1}. This demonstrates the potential of organic fertilizer, aviary manure for producing *Physalis*.

In this study, the increase in fruit production observed in plants that received organic fertilizer can be attributed to the availability of nutrients in a balanced way, without loss, either by leaching, fixation or volatilization, as in larger proportions in the mineral nutrient sources. According to Medeiros and Santos (2005), organic fertilizer provides a greater yield by providing sufficient quantities of nitrogen and potassium gradually, supplying, satisfactorily, the plant demand for these elements, which are essential for obtaining adequate production.

Conclusions

Through the results, local conditions, the experimental period, are favorable to the cultivation of the two species, and the organic fertilization, although did not differ in some parameters of chemical, provides higher plants, most mass and fruit in the standards set by international marketing and export standards, thus making possible scale production with utilization of materials (nutrients) that may be available on the property, without waste and with less risk of environmental contamination.

Conflict of Interests

The authors have not declared any conflict of interests.

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Variation of leaf area index of the forage sorghum under different irrigation depths in dynamic of cuts

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The determination of leaf area index is a variable of great importance for predicting productivity, especially when it comes to forage species where the desired product is the leaves. This study aimed to determine the variation of the leaf area index of forage sorghum when subjected to different irrigation depths in four culture cuts. Treatments consisted of six irrigation depths, these being 0, 60, 80, 100, 120 and 140% of the reference evapotranspiration. Seven evaluations of leaf area index (LAI) were performed, which were in the four cuts, specifically at 50, 80, 110 and 140 days after sowing (DAS), and also 15 days after each cut to evaluate the culture's resprouting, that is, at 65, 95 and 125 DAS. The delineation was randomized blocks, with six treatments and four blocks, totaling 24 experimental units. The experiment was conducted in Santiago-RS. Sowing was done on November 18, 2014. Using an irrigation system sprinkler, with one main row and six lateral rows, of PVC, being the irrigation levels differentiated by the nozzle diameter difference of sprinkler each lateral row. Samples of 0.5 linear meters per plot were collected in each of the seven evaluations, totaling 24 samples for evaluation. The analysis of leaf area index was performed using the computer program ImageJ. Significant results were found for the influence of the different irrigation levels on the leaf area index in the four cuts (recommended periods for grazing) evaluated, as well as for resprouting evaluations in the intermediate periods between cuts. Quadratic equations were adjusted for all the evaluation dates, where the 100 and 120% reference evapotranspiration levels alternated the largest LAI for all evaluations. The results also characterize that the second cut, or second grazing period of the irrigated forage sorghum, was the most productive, being it possible to recommend an increase in the animal load, or increase in grazing period in the area in this period. In this way, irrigation by sprinkling in the sorghum culture was characterized as an alternative for producers to increase leaf area index, and, consequently, crop productivity.

Key words: *Sorghum bicolor L. (Moench)*, irrigation management, pasture irrigation, sprinkler irrigation, production estimation.

INTRODUCTION

The leaf area index (LAI) is defined as the existing ratio of leaf area occupied by a population of any plant species, with the ground area, being extremely important in defining the productivity of a particular crop (Müller et al., 2005). Through the index, it is possible a better understanding of the relationship between environmental conditions and the dry matter accumulation in forages. In general, as the LAI increases, the growth ratio of forage species follow the same trend, reaching a value considered "optimum ", point from which there is a reduction in the growth rate (Molan, 2004).

According to Carnevalli et al. (2006), the LAI is an important parameter to be measured to conduct a proper management in cultivated pastures, through it is possible to determine the occupancy rate of the pasture or cuts performed, as well as the period of occupation or cutting intervals. Pasture management is extremely important for the success of the desired results, requiring special attention as regards the need to maintain leaf area to perform photosynthesis and reconcile with harvesting a large amount of high quality plant tissues, particularly leaves by grazing or pasture cuttings (Fagundes et al., 2001). Environmental factors such as temperature, light, soil fertility, genetic characteristics, pasture management, physiological plant age, and especially water availability, are intrinsically linked to the variation of leaf area index of forage species, leading to occurrence of variations according to the region and the rainfall incurred. Moreover, they are also critical in the new leaf area reconstitution capacity after cutting or grazing conditions, and this capability is crucial to the production and sustainability of pasture (Santos Jr. et al., 2004).

When subjected to some type of storm or stress, plants exhibit responses directly related to performance loss, the main constraint being water insufficiency condition. With the lack of water in their cells, the plants lack one of the primordial elements for the vegetative development, that is to say, foliar growth, causing in a smaller IAF and, insufficient for the reach of high productivities that characterize the viability of the costs of production (Larcher, 1986; Severino et al., 2004). The first plant strategy to adapt to drought conditions is the reduction of the aerial part due to the roots, limiting their ability to compete for light, by the decrease in leaf area, with consequent reduction in productivity (Nabinger, 1997). Considering that the irregularity of rainfall restricts the development of plants and that evapotranspiration of forage species is high at this time of year, there are usually periods of water insufficiency. In this way, the distribution of water in pastures through irrigation can improve productivity and profitability indexes by expressing a high leaf area index, compatible with production expectations, since, in the case of pastures, the main animal's power supply is the leaves (Cunha et al., 2007). The occurrence of water deficit in forage sorghum may lead to a reduction in the efficiency of the conversion of solar radiation to aerial mass, leading to a lower leaf and stem development, having a high influence on LAI (Dercas and Liakatas, 2007; Garofalo et al., 2011; Rinaldi and Garofalo, 2011). In addition, the adequate water supply of the forage sorghum crop, performed with irrigation shifts established through irrigation, increases the efficient use of water, with an increase in the leaf and stem mass increment, directly influencing LAI (Garofalo and Rinaldi, 2013).

In this context, sorghum has been characterized in cattle production system as a species used in animal feed due to high dry matter produced by the high number of leaves and their expansion, which can be offered by grazing or cuttings dynamic dynamic (Silva et al., 2015). However, for the crop to express their potential and achieve high levels of dry matter productivity, LAI must be high, where the main determining factor is water availability.

The availability of water for the crop be it through the occurrence of precipitation or supplement the evapotranspiration need through irrigation, is the alternative to food production in quantity and quality (Hefny et al., 2013). Thus, irrigation has been identified as an alternative to increase production in pastures, helping to reduce the effect of their seasonality. The response of dry matter forage production is directly related to climatic conditions, being of extreme importance the increased production caused by irrigation independently of the forage species, having the LAI as the main factor in increasing productivity in irrigated pastures (Vitor et al., 2009). Therefore, the objective of this study was to determine the variation of the leaf area index of forage sorghum when subjected to different irrigation in four crop cuts.

MATERIALS AND METHODS

The experiment was conducted in the agricultural year 2014/2015, in an experimental area located at Fazenda Liberdade, District of Tupantuba, municipality of Santiago, in the state of Rio Grande do Sul-Brazil. The area is located at latitude 29°09 '50' 'S, longitude 54º51' 32” W and altitude of 439 meters. Sowing of sorghum (*Sorghum bicolor* L., Moench) performed in a direct sowing system under crop residue, using cultivar Nutribem Elite, of Atlântica Sementes, on November 18, 2014. The sowing was done using one pull type mechanical seeder with spacing of 0.36 m between rows. For the establishment of the plant population were deposited to the ground about 15 seeds per linear meter of sowing, aiming at a final population of 330,000 plants.ha^{-1}. The climate in the region, according to Koopen’s scale (Moreno, 1961), is characterized as humid subtropical (Cfa), with an average temperature of 17.9°C annually. The average long-term rainfall is 1769 mm. The maximum, minimum and average temperature conditions at 7 day intervals occurring during the experiment are shown in Figure 1.

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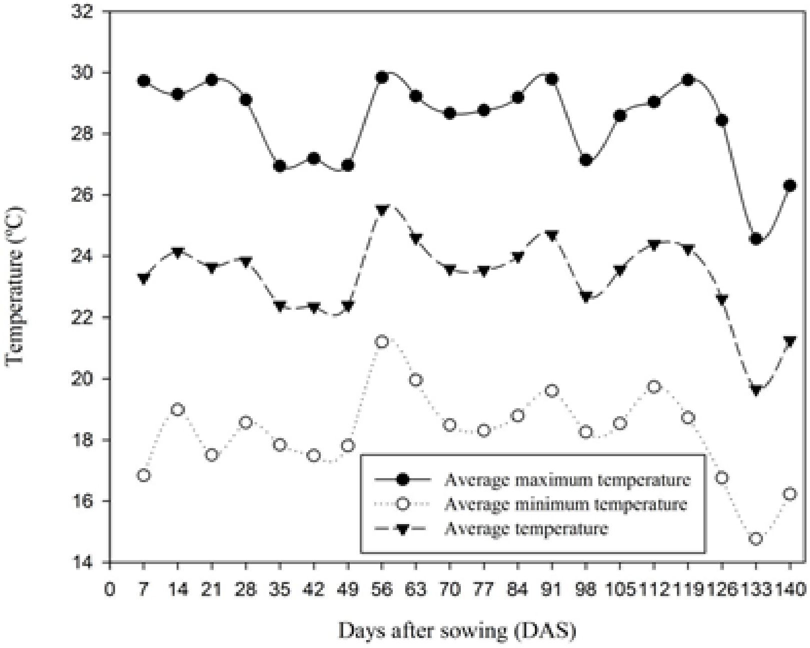

Figure 1. Average of maximum, minimum and average temperatures during the experiment.

The distribution of rainfall during the summer period is usually irregular, causing periods of drought stress to crops, because the atmospheric evaporative demand is high in the period, and the precipitations usually are not sufficient to meet crop needs (Nied et al., 2005). The rainfall and effective precipitation occurred during the experiment at 7 day intervals are shown in Figure 2. The soil used for the experiment is classified as Typical Dystrophic Red Latosol, which are deep to very deep soils and with the presence of a textural gradient profile, with horizon B (more clay) and horizon A (Streck et al., 2008).

The basic fertilization was done jointly with the sowing of the forage sorghum culture through a seeder-fertilizer, according to the interpretation of the soil chemical analysis, being deposited on the seeding row, below and beside the seeds. 250 kg.ha^{-1} fertilizers were applied with a commercial formulation of 5-20-20 nitrogen (N), phosphorus (P) and potassium (K), respectively. Nitrogen fertilization (N) or topdressing was carried out fractionally, based on the content of soil organic matter, which were applied 150 kg ha^{-1} of urea in each of the applications, which were at tillering and after each of the cuts made. Cultural practices relating to the application of fungicides, insecticides and herbicides were performed evenly for all treatments, covering the whole experimental area. Treatments consisted of six different water depths, evaluated in four blocks, totaling 24 experimental units, using a randomized block design. The irrigation depths used were 0, 60, 80, 100, 120 and 140% of the reference evapotranspiration (ETo). For the determination of ETo was used Penman-Monteith / FAO (Allen et al., 2006). Irrigation management was established with fixed irrigation shift of seven days, and irrigations were held whenever the effective rainfall during the irrigation interval of the shift did not meet the evapotranspiration demand of the crop. The determination of effective precipitation was calculated using the methodology proposed by Millar (1978).

It was used a conventional type sprinkler irrigation system comprising a main line measuring 60 meters and six fixed wings measuring 48 meters was used with all pipes of PVC system. The spacing of the wings was 12 m, connected with quick coupling. The nozzles were connected to these lines with spacing of 12 m and elevation of 1.5 m above the ground. The sprinklers used were the NAANDAINJAIN make, model 427 ½ " complete rotations. Differentiation of irrigation depths was performed by overlapping spray nozzles with different diameters where each of the six side rows received a nozzle diameter, namely: 4.0 mm × 3.5 mm; 3.2 mm × 3.0 mm and 2.8 mm.

We adopted the intermediate depth as 100% ETo, yielding after Christiansen uniformity test irrigation water and adjusted calibrated. Four crop cuts were performed at 50, 80, 110 and 140 days after sowing (DAS). For the evaluation of LAI were performed seven evaluations in fifteen day intervals, which were, at 50, 65, 80, 95, 110, 125 and 140 DAS. The first evaluation was performed 50 days after sowing (DAS), as the period recommended by the manufacturer of the seed used for the first cut of culture. The other evaluations were performed at intervals of 15 days, i.e., in the interim period between the previous and the next cut. It is noted that the cuts were made in accordance with the recommendations of the manufacturer of the seeds, the first cut in a wider range of days due to the culture of the sorghum presenting toxicity to animals in the early stages of development, having in its composition high levels of tannin and hydrocyanic acid. Therefore, it is recommended that the first cut be made 50 days after sowing, and the other cuts at intervals of 30 days.

Samples of 0.5 linear meters per plot were collected in each of the seven evaluations, totaling 24 samples for evaluation. The analysis of leaf area index was performed using the computer program ImageJ, where it analyzes photographs of leaves present in the sample, and to take photographs, the leaves were laid on a white background, where it was placed a square object of known size and photographed. The computer program ImageJ through the contrast between the paper and the white background and the knowledge of the proportion of area of the known object, determined the leaf area of the sample as Figure 3. As the row spacing was 0.36 linear meters and collections were made at 0.5 linear meters, the sample represents0.18 m2, making it possible to obtain the Leaf Area Index (LAI) existing in a square meter. The

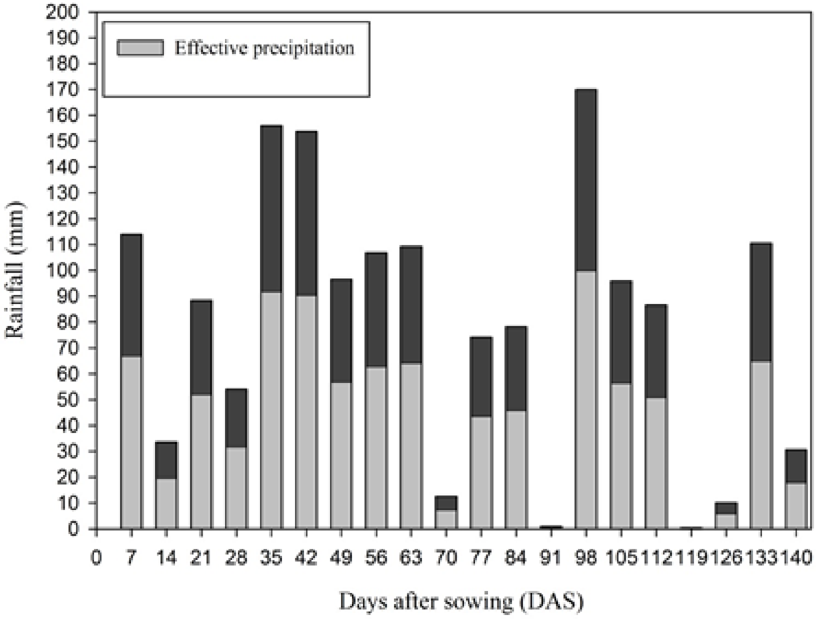

Figure 2. The rainfall and effective precipitation occurred during the experiment.

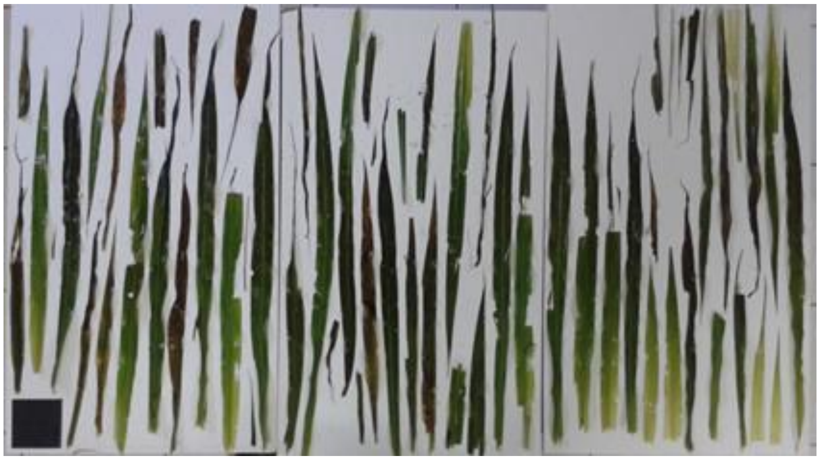

Figure 3. Photography for determining the leaf area index of the forage sorghum culture.

data obtained referring to the leaf area index found for each of the assessments carried out over the days after sowing (DAS) were evaluated statistically by variance-ANOVA and subsequently the individual effects of the treatments were evaluated by regression analysis using software SISVAR 5.3 (Ferreira, 1998), considering the statistical tests at 5% probability of error and for the preparation of graphic images was used SIGMAPLOT 11.0 software.

RESULTS AND DISCUSSION

During the experiment period of implementation, within the scope ranging between November 18, 2014 and April 6, 2015, that is, during the four cuts made in the sorghum crop, the amount of rainfall was 883 mm. However, since the experiment is divided into cuttings period, precipitations within each cutting interval were quite varied, and, in certain occasions it was required to replenish the evapotranspiration demand of the crop through irrigation. In addition, according to Millar (1978), part of the total rainfall occurred is lost, called lost by rainfall surface run-off. This fraction of the lost precipitation can be estimated according to the type of soil, soil slope and the cultivation condition. For the location of the study, the fraction of precipitation lost by runoff used is 30% of the total precipitate. Thus, the actual rainfall during the study conduct was 618 mm.

Table 1. Effective precipitation, reference evapotranspiration (ETo) and irrigation depth applied in the seven-day irrigation schedule established.

	DAS	EP (mm)	ETo (mm)	Irrigation levels (mm) – % ETo				
				60	**80**	**100**	**120**	**140**
1º cut	7	46.9	35.3	0.0	0.0	0.0	0.0	0.0
	14	13.9	30.4	8.1	13.2	16.5	19.8	23.1
	21	16.4	43.1	16.0	21.3	26.7	32.0	37.4
	28	12.3	31.0	11.2	14.9	18.7	22.4	26.2
	35	64.3	31.8	0.0	0.0	0.0	0.0	0.0
	42	63.3	22.1	0.0	0.0	0.0	0.0	0.0
	49	39.8	32.8	0.0	0.0	0.0	0.0	0.0
		256.9	**226.5**	**35.3**	**49.4**	**61.9**	**74.2**	**86.7**
2º cut	56	43.0	21.8	0.0	0.0	0.0	0.0	0.0
	63	44.9	22.6	0.0	0.0	0.0	0.0	0.0
	70	5.2	33.1	16.7	22.3	27.9	33.5	39.0
	77	30.5	29.1	0.0	0.0	0.0	0.0	0.0
		123.6	**106.5**	**16.7**	**22.3**	**27.9**	**33.5**	**39.0**
3º cut	84	32.2	32.2	0.0	0.0	0.0	0.0	0.0
	91	0.4	32.5	19.2	25.7	32.1	38.5	44.9
	98	70.0	21.1	0.0	0.0	0.0	0.0	0.0
	105	39.5	28.4	0.0	0.0	0.0	0.0	0.0
		142.1	**114.1**	**19.2**	**25.7**	**32.1**	**38.5**	**44.9**
4º cut	112	35.7	27.0	0.0	0.0	0.0	0.0	0.0
	119	0.1	29.9	17.9	23.8	29.8	35.8	41.7
	126	1.2	28.9	16.6	22.2	27.7	33.2	38.8
	133	45.5	13.9	0.0	0.0	0.0	0.0	0.0
	140	13.2	16.9	0.0	0.0	0.0	0.0	0.0
Total		**95.7**	**116.6**	**34.5**	**46.0**	**57.5**	**69.0**	**80.5**
	140	**618.3**	**563.7**	**105.7**	**143.4**	**179.4**	**215.2**	**251.1**

In Table 1 is shown the effective precipitation (EP) the reference evapotranspiration (ETo) and the total water applied in each irrigation levels on each cut made at intervals of seven days due to the irrigation schedule. As shown in Table 1, the variation in precipitation incurred within the seven-day interval irrigation schedule was high, where, at certain periods, there was no need for water replenishment through irrigation, and other times the supplement of water was necessary.

According to Von Pinho et al. (2007), the water demand required for normal yields compared to the culture average, provided by an elevated LAI of the sorghum is 380 to 600 mm. Thus, the effective precipitation of 618 mm incurred would be enough to meet the water requirement of the crop.

However, in the case of forage sorghum, conducted in dynamic cuts, this data becomes quite relative, as the variation of water regime is characterized as essential, where the variability over the days and cuts may compromise development of crop LAI, once that, if the occurrence of rainfall regime is poorly distributed, it will result in low growth and senescence of leaves, then being required an evaluation of rainfall through specific irrigation schedules, so that there is no impairment of LAI and consequently in the crop yield. Previous studies carried out in the same experimental site, already point to the occurrence of dry spells in previous years, that is, water deficit periods for the culture, causing a decrease of leaf area index and consequently the final crop yield of corn, soy and beans, as well, results show increment of leaf area index in these different species depending on the variation of the replacement of the water requirement through different irrigation levels (Parizi et al., 2009; Gomes, 2011).

During the trial implementation period, the distribution of rainfall was irregular, requiring replenishment of evapotranspiration demand through irrigation. Seven supplemental irrigations were required, three in consecutive weeks in the interval between the sowing and the first cut, one between the first and the second cut, one between the second and third cut and two, in consecutive weeks, between the third and fourth cut.

Although the experiment was conducted in a year with above average rainfall for the period, and some irrigations were performed interleaved way with the rainfall, there was influence of irrigation on the variation

Table 2. Values of leaf area index found for the different irrigation depths applied throughout the forage cycle of the culture.

irrigation depths (%ETo)	Days after sowing (DAS)						
	50*	65*	80*	95*	110*	125*	140*
Without irrigation	2.808	1.482	3.280	1.192	2.768	0.461	1.320
60	3.868	2.369	4.449	1.606	4.292	0.562	1.570
80	4.183	2.526	4.526	1.930	4.465	0.622	1.860
100	4.432	2.647	4.715	2.091	4.684	0.706	2.222
120	4.260	2.544	4.806	2.144	4.783	0.738	2.124
140	4.188	2.334	4.503	2.076	4.562	0.715	2.090

*Significative at 5% of probability by test F.

Table 3. ANOVA for the influence of irrigation depths on LAI of forage sorghum in the DAS.

Days after sowing (DAS)	Sum of square (SQ)	Middle square (MS)	Fc	Pr>Fc
50	6.984900	1.396980	39.712	0.0000*
65	3.611871	0.722374	33.629	0.0000*
80	6.189033	1.237807	33.832	0.0000*
95	2.778033	0.555607	36.367	0.0000*
110	11.227921	2.245584	125.563	0.0000*
125	0.232783	0.046557	16.048	0.0000*
140	14.526950	2.905390	76.330	0.0000*

*Significative at 5% of probability by test F.

of the leaf area index in different depths applied. To determine the treatments were used and adjusted calibrated irrigation water according to Christiansen uniformity coefficient being also 0, 60, 80, 100, 120 and 140% of the reference evapotranspiration (ETo).

In Table 2 is shown the leaf area index found in different water depths applied in different days after sowing (DAS) evaluated in 15 days interval from the first cut. As shown in Table 2, there was great variation in leaf area index for all valuation dates, and between the irrigation depths applied, where, in general, there was an increase in the LAI as the increase of the depth up to a point where excess water caused the decrease of the LAI.

In Table 3 is shown the ANOVA for the influence of irrigation depths on LAI of forage sorghum in the DAS. For the first valuation date, that is, the first cut of culture, held 50 days after sowing, the LAI ranged from 2.808 in non-irrigated plot to 4.432 in water depth of 100% of ETo, as shown in the Figure 4.

The results obtained for the variation of the LAI in the first cut (50 DAS), it is possible to observe as shown in Figure 4, the importance of water for growth and leaf development in the forage sorghum crop, where the lowest value was obtained in non-irrigated plot with 2.808, featuring that there was water stress, and that development was not according to the potential of culture, once that the LAI for the plot with 100% replacement of ETo was 4,432. According to Jaleel et al. (2009), the water stress is responsible for cell growth inhibition, resulting therefore in less LAI, because the plants under water stress undergo changes in various physiological processes such as photosynthesis and respiration, resulting in the lower leaf expansion.

The results found are opposite to those obtained by Marcelino et al. (2003), which analyzed the leaf area index in Tifton 85 culture, cultivated under different water stress in the soil, found no significant statistically difference for the LAI in assessment carried out in the first cut. However, although there was no significant statistical difference, the values found are of increased LAI as increased irrigation to values close to 100% of replenishment, declining soon after, being consistent with those found for the first cut of forage sorghum. On the other hand, the results obtained of increase of the LAI in different depths of water in relation to the non-irrigated treatment are similar to those obtained by Andrade et al. (2005). The authors, in study realized analyzing the growth of grass-elephant "NAPIER" fertilized and irrigated, observed increased leaf area index in the irrigated plot, with an average of 8.27 compared to the average of the non-irrigated treatment 7, 76. According to Müller et al. (2005), those of the LAI increased results with the use of irrigation can be explained by the significant increase in CO_2 assimilation surface and photosynthetic active radiation interception, increasing respiration and increasing the plant’s consumption of the water available.

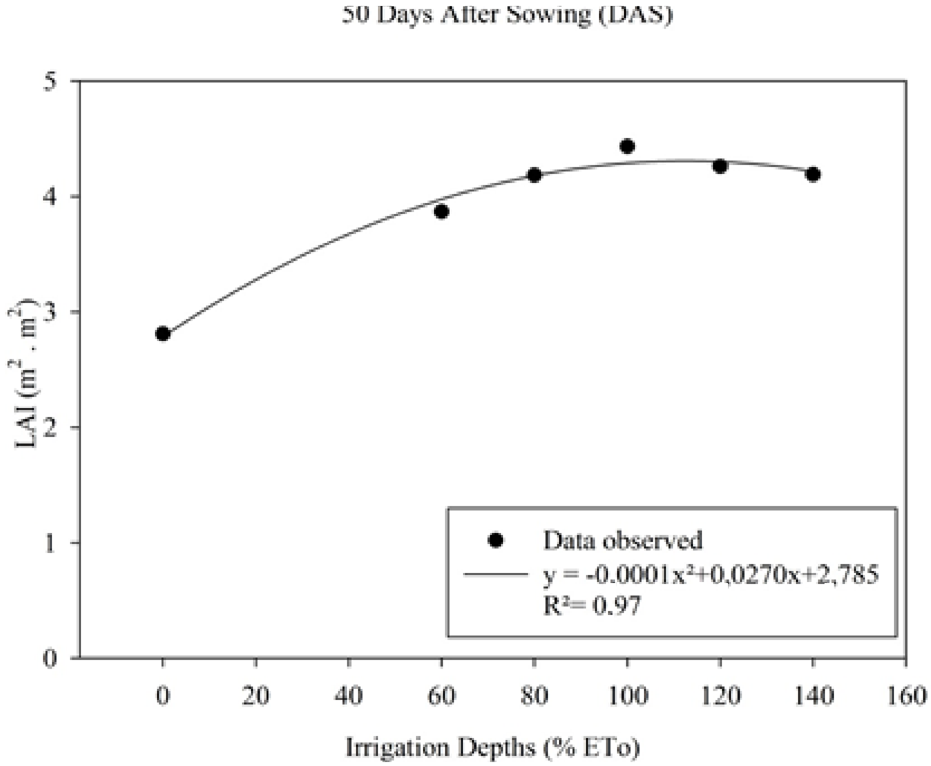

Figure 4. Leaf area index of the forage sorghum culture, at 50 DAS, in the different irrigation depths applied.

Ferraz et al. (2012) highlights that through the greater availability of water, plants increase gas exchange between plant and atmosphere, causing expansion of plant tissues and shoot development. For the evaluation of the LAI in the second cut, two collections were made, the first 15 days after the first cut, or 65 DAS, in order to verify the culture resprouting behavior and the importance of water on the new leaf area index. The second, 30 days after the first cut due to the fact that the recommended period for cutting or grazing entry for the second time for the forage sorghum crop.

The results for the variation of the LAI in different irrigation levels at 65 and 80 DAS are shown in Figure 5. As shown in Figure 5, the influence of the different depths of irrigation applied followed the same trend found in the first cut to 65 DAS and the second cut (80 DAS) with a quadratic response and increased leaf area index with increasing irrigation depth up to a point where the excess water caused a drop in rated parameter. In the evaluation at 65 DAS, or 15 days after the first cut, the leaf area index ranged from 1.482 in non-irrigated plot to 2.647 in the depth of 100% of ETo, thus demonstrating the importance of water to the establishment of new leaf area after the cut, as the case of forage species, the leaves are the main component for animal feed.

For the second cut, performed at 80 DAS, or 30 days after the first cut, the behavior followed the same trend, with adjusted quadratic equation, where the values found ranged from 3.280 in the treatment without irrigation to 4.806 in treatment with replenishment 120% of ETo. It is noted that the second cut was the one that had the largest LAI, the main cutting for the forage sorghum culture. These results are attributed to the period of the year favorable to the growth and development for the forage sorghum culture in State of Rio Grande do Sul optimum after the first cut, as for sowing done in November, the growth period after cutting is in the months of December and January, a period where there are high temperatures and with adequate water supply and photoperiod, supplies the main growth and development needs of the forage sorghum culture.

Corroborate with the results found in the work Zwirtes et al. (2015), that evaluating the morphological characteristics and productivity of sorghum plants submitted to deficit irrigation, achieved increment of 15% in the LAI in treatment 100% of ETc with LAI of 7.96 cm from the treatment of 25% ETc with 6.04 cm, at 60 days after emergence. With these results, it is clear that the change in water depth, variation provided the LAI with increased rate as the increase in irrigation depth. As the results found by Zwirtes et al. (2015), the obtained in this study had an increase in the LAI with the increased level of irrigation, however, due to the standardization of cuts made, the IAF's maximum obtained were below those

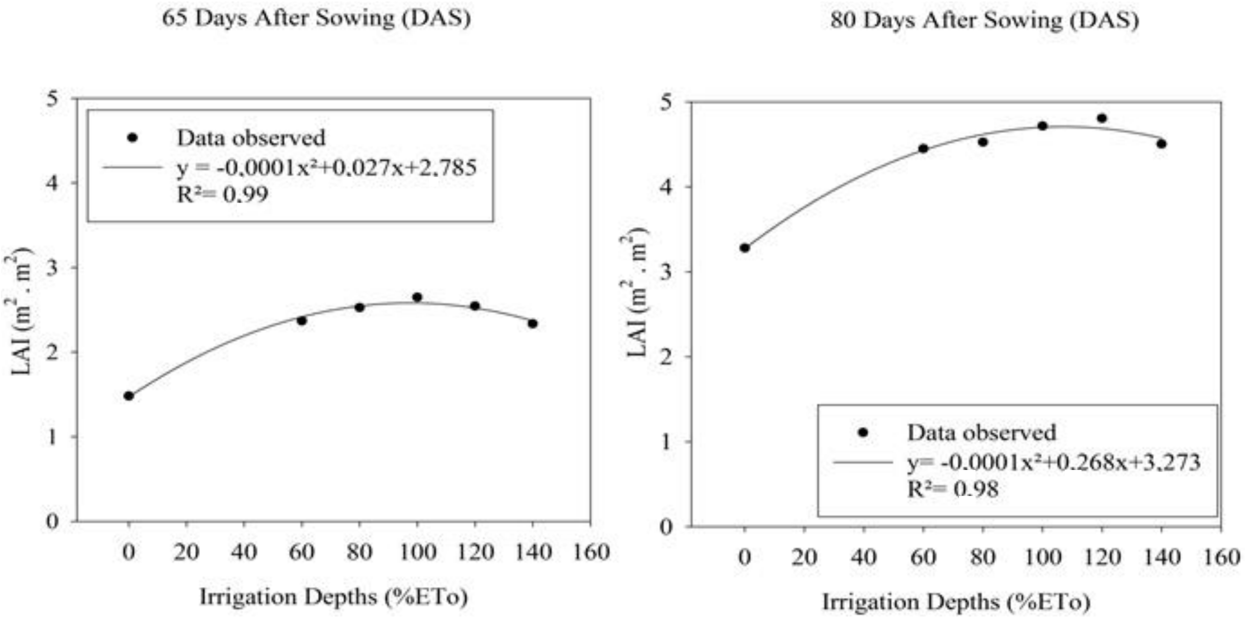

Figure 5. Leaf area index of the forage sorghum culture, at 65 DAS and the second cut (80 DAS) in different irrigation depths applied.

found by the author cited, where the highest levels were found at 80 DAS, with 4.80, at depth 120% of ETo.

Also corroborate Viana et al. (2005), that working with the production of biomass and LAI in forage grasses in irrigated and dry during the dry season, obtained increase in LAI of 25% in the system irrigated compared to non-irrigated for forage species studied, they are the grass Tanzania, Marandú and Pioneer. The authors cited, found rates ranging from 1.52 to 4.16, being close to the minimum obtained at 65 DAS in treatment without irrigation with 1.48, and also to the maximum obtained at 80 DAS in the treatment with 120% of ETo with 4.80.

According to Peiter and Carlesso (1996), the results of reducing the LAI, caused by water stress, is an irreversible fact for the plant. The authors point out that this behavior is the morpho-physiological adaptation of the plant, especially when it occurs during the growth and development of the sorghum crop, causing decreased absorption in interception and photosynthetically active solar radiation.

For the evaluation of the LAI in the third cut, two samples were collected, the first 15 days after the second cut, that is, 95 DAS, in order to verify the crop resprouting behavior and the importance of water on the new index leaf area and the second, 30 days after the second cut due to the fact that the recommended period for cutting or grazing entry for the third time for the forage sorghum crop. The results for the variation of the LAI in different irrigation levels at 95 and 110 DAS are shown in Figure 6.

As seen in Figure 6, the influence of the different depths of applied irrigation followed the same trend found in previous evaluations, adjusted quadratic equation, that is, quadratic, both for 95 and for 110 DAS. For the two evaluations, there was an increase in LAI with increasing water depth up to a point where the excess water caused a drop in rated LAI. For the evaluation carried out at 95 DAS, or 15 days after the second cut, LAI ranged from 1.192 to 2.144 non-irrigated treatment in the treatment with replacement blade 120% ETo.

These results show that with adequate water supply, completion of the third cut period recommended for the forage sorghum crop in the Midwest region of Rio Grande do Sul is possible because the LAI in larger blades kept values close to those obtained in the first resprouting after the first cut (65 DAS).

For the third cut, held at 110 DAS, or thirty days after the second cut, the behavior followed the same trend, with adjusted quadratic equation, where the values found ranged from 2.768 in the treatment without irrigation to 4.783 in treatment with replenishment 120% of ETo. It is noted that the second cut was the one that got the largest LAI, the main cutting for the sorghum crop.

Favarin et al. (2002), points out that the study of variation of leaf area index allows to estimate the variation of the water requirement of culture, being an extremely important tool to define the best management strategy for irrigation. Similarly, the authors state that the variation of water availability is responsible for changes in the LAI and consequently productivity, a fact, checked on the results found.

According to Andrade et al. (2005), the evaluation of growth of forage species makes it possible to identify the characteristics of plants, associated with the adaptations

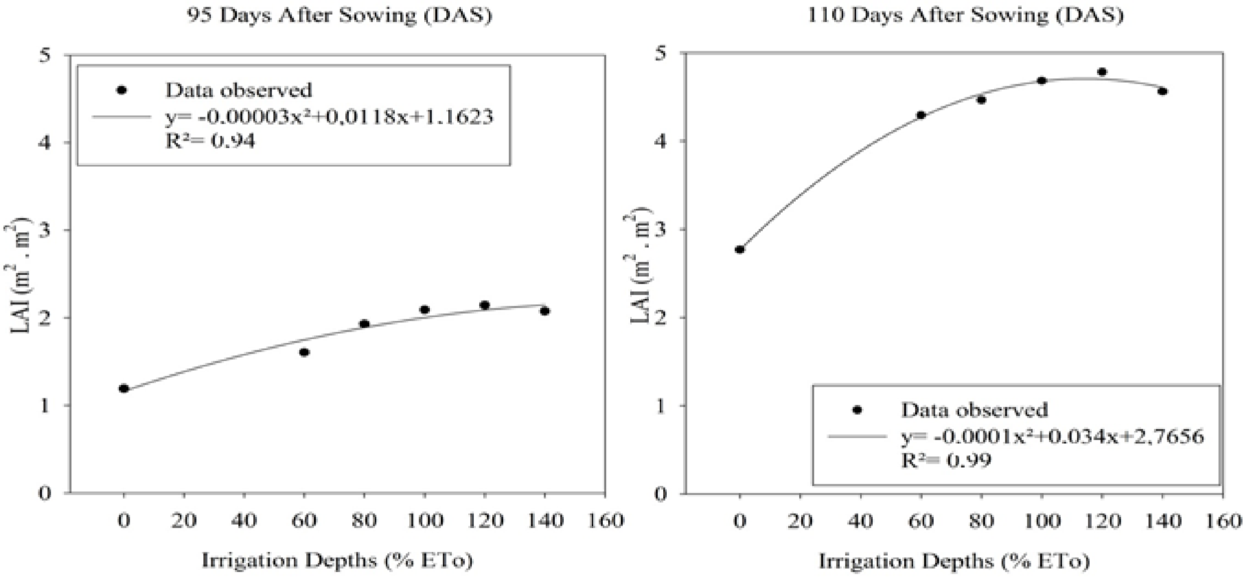

Figure 6. Leaf area index of the forage sorghum culture, at 95 and 110 DAS, in different irrigation depths applied.

to adversities caused by stress, as well as their potential for optimal growth conditions. The results are consistent with those obtained by Fagundes et al. (2006), who observed reduction in grass-braquiária IAF in the period with lower availability of water.

Also corroborate the results, obtained by Coutinho et al. (2015), who observed that the LAI decreased with increasing irrigation interval ranging from 0.1 to 10-day watering part, 1.1 at 2 days irrigation schedule work with the culture Capim -buffel. In contrast, the results obtained by Cunha et al. (2008), working with the relation between the spectral behavior, leaf area index and dry matter production in Tanzania grass under different levels of irrigation and nitrogen doses did not achieve significant results for leaf area index for irrigation depths of 0, 30, 70, 100 and 150% of field capacity, attributing these results the occurrence of heavy rainfall during the experiment.

For the evaluation of the LAI in the fourth cut were two collections, the first 15 days after the third cut, that is, 125 DAS, in order to verify the crop regrowth behavior and the importance of water on the new LAI and the second, 30 days after the third cut due to the fact that the recommended period for cutting or grazing entry for the fourth time for the forage sorghum culture. The results for the variation of the LAI in different irrigation levels at 125 and 140 DAS are shown in Figure 7. As seen in Figure 7, the influence of the different depths of irrigation applied followed the same trend found in previous evaluations, adjusted quadratic equation, or quadratic, for both the 125 and 140 DAS. For the two evaluations, there was an increase in LAI with increasing water depth up to a point where the excess water caused a drop in the LAI.

It should be noted that in all evaluation dates made the fourth cut was where we found the lowest LAI amounts. This behavior is due to the fact that the period of growth and development of regrowth after the previous cut, match the decline in temperatures in the state of Rio Grande do Sul, and because sorghum is extremely sensitive to low temperatures, the LAI was smaller than the other evaluation periods.

According to Diniz (2010), sorghum has high capacity in average temperatures over 21°C, and the productive aspects severely hurt by lower average temperatures, a fact which was verified in this study. Some authors point out that although the entire climate complex exercise influence on the growth and development of plants, the temperature is the dominant factor (Berlato and Sutili, 1976; Berlato et al., 1984).

For the evaluation conducted at 125 DAS, or 15 days after the third cutting, ranged from 0.461 LAI without irrigation treatment at 0.738 in treatment with replenishing depth of 120% ETo. These results demonstrate that with proper water supply, there is variation in the LAI, however, yields found are low compared to other sprouts after the previous cuts. For the fourth court, held to 140 DAS, or thirty days after the third cut, the behavior followed the same trend of previous cuts.

The equation was adjusted quadratic where LAI values found ranged from 1.320 in the treatment without irrigation to 2.124 in treatment with replenishment of 120% of ETo. It is noted that the LAI values found for the fourth court were much lower compared to previous cuts. The highest LAI found in the blade replacement of 120% of ETo, or 2.124 in the fourth cut is less than half of the observed value for the second cut, the most productive, the depth of 100% ETo or 806.

The factor responsible for this decrease is the drop in

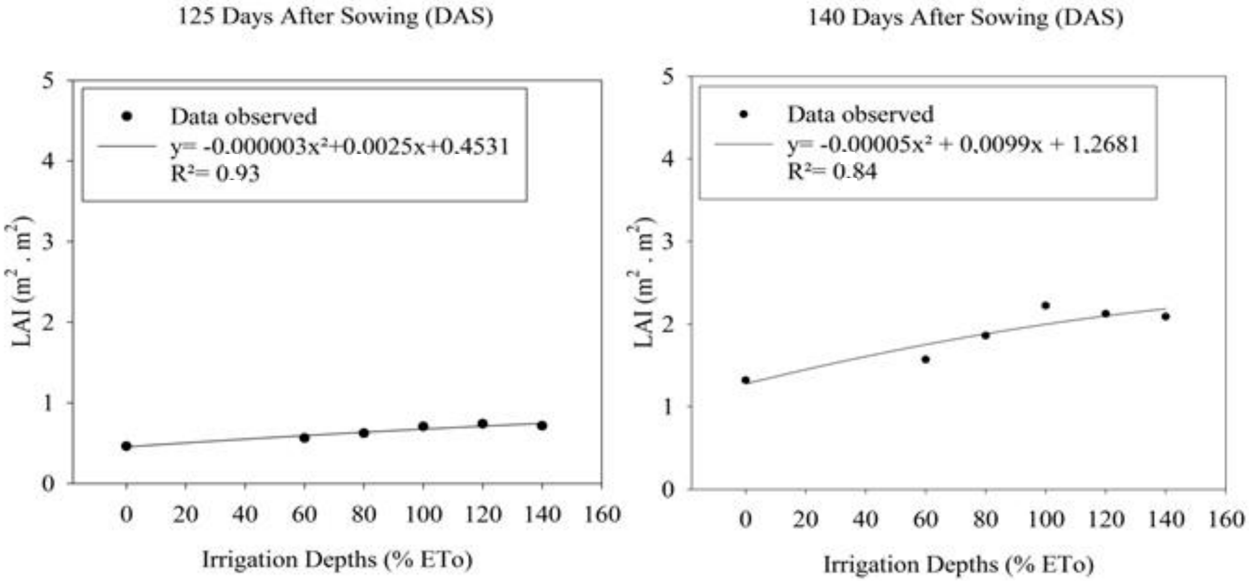

Figure 7. Leaf area index of the forage sorghum culture, at 125 and 140 DAS, in different irrigation depths applied.

temperatures in April in the state, that limit the growth and development of culture, making it impossible for producers in the region due to costs and the small return, forage sorghum cultivation is recommended only until the third cutting for the region.

The results are opposite to those obtained by Marcelino et al. (2003), which analyzed the leaf area index in Tifton 85 culture, grown under different water stress in the soil, found no statistically significant difference for the LAI evaluation performed on the fourth cutting. However, although there was no statistical difference, the values found are of increase of the LAI as increased irrigation to values close to 100% of replacement, declining soon after, being consistent with those reported for the fourth cutting of forage sorghum.

Regarding the total dry mass production found due to different levels of irrigation in the sum of the four cuts the same is represented in Figure 8. From the results seen in Figure 8, it was possible to see the effect of different irrigation levels on increasing the amount of total dry matter produced in the sum of the accumulations of the four cuts made.

Thus, it was observed that the total productivity of the sum of the four cuts maintained the same trend obtained in the individual analysis of the cuts, where the highest yields were found in depths with higher levels of irrigation, which were in the one of 140 % of the ETo with 13,690 kg.ha^{-1} and then the one of 120% with 13,058 kg.ha^{-1}. The variation between the irrigation level yields with higher means, that is, replacement of 140% of ETo and the non-irrigated level was 80.36%.

And according to the recommendations by Atlântica seeds, producer of the seeds used, yields can be up to 100% higher when using irrigation in connection with flooded farming agriculture systems. However, the results of increase found in the production of irrigation levels with higher productivity compared to the non-irrigated one are attributed to the year of the work, which presented high total precipitation and only seven irrigations were necessary throughout the period. And in years with lower precipitation it is possible to find better results for the use of sorghum irrigation.

Vale and Azevedo (2013), evaluating the productivity and quality of elephant grass (*Pennisetum purpureum*) and sorghum irrigated with desalinated ground water and waste, have obtained a productivity of 14,500 kg.ha^{-1} of dry matter in treatments irrigated with groundwater, while in this study the highest total production of dry matter in the sum of the production of the four cuts made was in the level of 140% of the ETo with 13,680 kg.ha^{-1}, that is, close to what the authors found.

Another work which corroborates the results obtained is the one by Zwirtes et al. (2015), who, in evaluating morphological and physiological characteristics and productivity of sorghum plants submitted to deficit irrigation, have found higher dry matter yield in the level of 100% of the ETo with 13,785 kg.ha^{-1}, being close to the 13,680 kg.ha^{-1} found in the level of 140% of the ETo.

Skonieski et al. (2010) also contribute, when working with double purpose sorghum, in finding dry matter production of 13,006 kg.ha^{-1}, while for sorghum they obtained a productivity of 17,527 kg.ha^{-1}. Cunha et al. (2010), evaluating the dry matter production in 23 genotypes of sorghum, have obtained an average of 15,703 kg.ha^{-1} of dry matter production, with productivity of the genotypes ranging from 13,467 to 26,100 kg.ha^{-1}, and the average productivity close to the maximum found in this study which was 13,680 kg.ha^{-1} in the level of

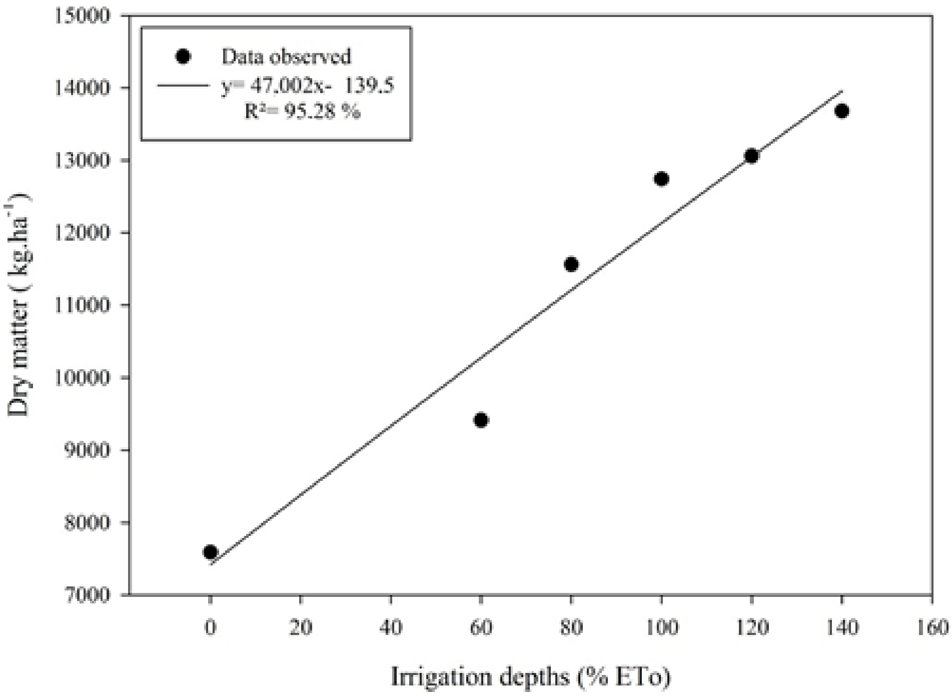

Figure 8. Total dry matter in different irrigation depths in the sum of the four cuts made.

140%.

Tabosa et al. (2002) also contribute for the results found in the study in an experiment with sorghum cultivars in different agroecological environments. They have obtained varied yields of dry matter in water stress conditions, with an average production of dry matter ranging from 3,320 to 15,860 kg.ha^{-1} for cultivar 02-03-01 and 2,820 to 20,130 kg.ha^{-1} for SF-25, grown in five different locations in the Brazilian state of Pernambuco.

As for Silva et al. (2005), in a study on the adaptability and stability of forage sorghum cultivars at different times of the year, have found a yield ranging from 9,423 to 13,426 kg.ha^{-1}. As for Oliveira et al. (2005), working on the agronomic characteristics of sorghum cultivars, have found yields ranging between 14,220 and 16,380 kg.ha^{-1}, with an average of 15,170 kg.ha^{-1}.

Regarding the increase in production caused by the different irrigation levels tested in relation to the non-irrigated portion, several studies show increased production results with increasing irrigation level use. Melo (2006), also working with sorghum, has obtained 57.9% production increase by irrigation with an irrigation level of 100% of field capacity in relation to the level of 25%, whereas in the present study the increase of the level of 100% of the ETo in relation to the non-irrigated one was 67.87%, with an increase of 5,184.3 kg.ha^{-1}, and, for these authors, these results are due to an adequate water supply in time of need.

Therefore, the results found by the different authors and mentioned show that the results obtained in this study are close to the studies carried out with the crop. They vary within the different irrigation levels and the productivities found within the time ranges reported in the literature. Statistically significant differences are found for the effect of irrigation levels on the yields found.

The results found for the variation of dry matter production per hectare in the different irrigation levels over the four cuts in forage sorghum crop show the importance of water for the plant metabolism. The dry matter production increase per hectare in all evaluations carried out in the study may be explained due to the occurrence of certain factors.

For Pimentel et al. (2016), for proper growth and development of forage species, the correct water supply is one of the essential factors for the growth and development of leaves and stalks. With the correct water availability there is an increase in the exchanges between the environment and the plant, increasing photosynthesis and thus the growth of plants, a fact that can be observed in this study with the results obtained from the production increase in higher irrigation levels.

Bergamaschi et al. (2004), working with corn crops, have attributed the occurrence of water deficit to a reduction in the dry matter production. According to the authors, the damage caused by water deficit within the plants is higher than perspiration, suggesting that in addition to the direct effect of stomatal resistance to gas diffusion, photosynthesis is also affected by the increase in leaf temperature due to the stomatal closure. Also, leaf expansion is also reduced (Lopes et al., 1986) and this process is more sensitive than the stomatal movements and photosynthesis, affecting cell growth and being considered one of the most sensitive processes to water deficit, with the cell division and expansion directly inhibited by water stress, a fact which may have led to growth restriction (Sausen, 2007).

Bengough et al. (2006) and Nascimento (2008) highlight that water stress can be characterized as an adverse condition which inhibits normal functioning and the well-being of biological systems. According to these authors, when undergoing stress, plants receive a series of signals in their receptor cells that trigger responses in an attempt to maintain production. The effect of changes caused or imposed to plants depends on the ability to adapt to this new condition. However, damage usually occurs on productivity (Silva et al., 2012), a fact seen in the levels with lower water replenishment for the four stages of evaluation of this work.

The results of the work can still be explained by the stoppage of the growth and expansion of plants submitted to lower irrigation levels than necessary, together with high temperatures by the plant development period. Ferraz et al. (2012) point out that through the greater availability of water, plants increase gas exchange between plant and atmosphere, causing expansion of plant tissues and shoot development, especially leaves, which are essential when it comes to pasture dry matter.

According to Pimentel et al. (2016), another factor that could explain the results is the change in soil moisture which, when insufficient, entails a conflict between water conservation by the plant and CO^2 assimilation rate for carbohydrate production. In prolonged periods of water stress, even the most adapted plants undergo the consequences of this stress, resulting in lower growth, and consequently lower dry matter yield per hectare for forage species.

Chaves et al. (2003) also contribute to the results found in the work, relating the growth, development and forage productivity of plants, maintaining the hydration of the cells, as this would be maintaining the metabolic activities of the tissue and the integrity of cellular structures, a fact that is severely compromised in times of insufficient water availability. Another fact that may explain the lower production of dry matter in the portions with less replacement than necessary is abortion and death of leaves in forage species (Araya et al., 2011; Valle et al., 2009).

Santos et al. (2013) have found that in water stress conditions there was an increase in dead material in leaves of *B. brizantha cv. Marandu* and consequently decrease in total leaf area and dry matter productivity as mechanisms used to reduce water consumption and balance their water relations in conditions of water stress.

Mahajan and Tuteja (2005) characterize the dry matter decreased productivity per hectare of species when submitted to water stress not only to photosynthesis limiting intracellular CO^2, but also the cellular damage to the photosynthetic apparatus due to accumulation of reactive oxygen species. This takes place due to the decline of CO^2 in the cells, resulting in a marked reduction of components within the electron transport chain that transfer electrons of Photosystem I to oxygen, generating their reactive species, harmful to cells, thus causing stoppage of plant growth.

According to Lawlor (1995), the results found in this study can be explained by the decrease in photosynthetic rate caused by the drought in the levels with lower water replenishment through irrigation. The possible reasons for this decrease in photosynthetic rates when plants are submitted to drought stem from the limited diffusion of CO^2 into the intercellular spaces of the leaf as a result of stomatal reduction and impaired metabolism due to direct inhibition of biochemical processes by loss of water in cells.

Farooq et al. (2009) also relate the decreased productivity of forage species when submitted to water stress to the fact that the plant extracts water from the soil only when the water potential of its roots is more negative than that of the soil solution and the absorption rate is higher as the absorption surface of the root system is greater. The water potential gradient across the soil-plant-atmosphere system constitutes the driving force to transport water through the plant and this relationship is compromised and the root system supply to the shoots is hindered under water stress conditions.

Conclusions

The use of irrigation exerted great influence on the variation of the leaf area index in all evaluation dates performed. All evaluations showed quadratic behavior, which, with increasing applied irrigation depth, an increase of LAI to some extent, where the excess water caused drop in LAI.

The 100 and 120% of ETo water depths were those that provided the best results, characterizing the crop response to water increase provided through irrigation.

The Sorghum crop presented high LAI when irrigated, characterizing the irrigation of forage Sorghum as an alternative to the farmers and ranchers of the Central-West region of Rio Grande do Sul-Brazil.

The irrigation sprinkler is presented as an alternative for producers in the Midwest region of Rio Grande do Sul, since its use has provided high LAI In cultivated pastures increase compared to non-irrigated plot.

The LAI values found indicate that for sowings in November in the state of Rio Grande do Sul, indicate that it is possible to perform three times cutting or grazing of forage sorghum, because after, the occurrence of lower average temperatures there are prejudices of LAI and hence of crop productivity.

There was a great increase of dry mass production in the plots with larger irrigation slides, characterizing that the larger leaf area indices found resulted in higher yields.

Conflict of interest

The authors have not declared any conflict of interest.

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Mathematical models to estimate leaf area of citrus genotypes

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Mathematical models were developed, using 22 different genotypes of citrus, to estimate leaf area. The information of the relationship between leaf length and width $(L/W)^2$ for simple leaf blade form (eliptic, ovate, obovate, lanceolate); and length of the three folioles $(L_2 + L_3)/L_1$ for a compound leaf (trifoliate leaves), was used with the purpose to separate group of similarities of leaf blade form and promote high accuracy of estimate. The best models presented an excellent precision with errors varying from 1.2 to 6.2 (%) and r^2 higher than 0.95 for the majority of the models tested. Considering a single leaf blade, the linear model ($Y = \beta \cdot L \cdot W$) presented the lower mean deviation and lower square deviation. For the compound leaves, the potential models are simple to use, since use only the information of length of central foliole L_1 ($Y = \beta L_1^{\mu}$), although the use of linear models gave the best precision, as observed by using the model $Y = \beta . L_1 . W_1$. Furthermore the model might be used as a single model independent of the relation $(L_2+L_3)/L_1$: $\{Y=\beta\cdot(L_1\cdot W_1 + L2\cdot W_2 + L_3\cdot W_3), r^2 = 0.98\}$.

Key words: *Citrus*, compound leaf, hybrids, leaf area modeling.

INTRODUCTION

Leaf area is a variable of extreme importance to physiological and agronomic studies, such as vegetable growing, interception of light, remote sensing, photosynthetic efficiency, absorption of carbon, evapotranspiration, absorption of agrochemicals by the leaves, assessment of pest attack and irrigation management (Coelho Filho et al., 2004, 2012; Guimarães et al., 2013; Griffith et al., 2011; Lopes and Pinto, 2005; Oliveira et al., 2013; Pereira et al., 2009; Silva et al., 2008; Stuckens et al., 2009).

Among the methods to estimate leaf area, mathematical models based on measures of biometric variables (leaf width and length) are widely used for various species of plants (Serdar and Demirsoy, 2006) and can be applied in studies of many types (Ramirez and Zullo Júnior, 2010; Bu et al., 2013; Coelho Filho et al., 2013; Padrón et al., 2016; Silva et al., 2008; Yi et al., 2010). However, due to the genetic variability for such characteristics, further studies and specific equations for each genotype are needed within a given species (Malagi

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Table 1. Genotypes used for leaf area estimation according to the leaf blade form.

	Single leaf (elliptical, ovate, obovate and lanceolate)	Compound leaves (trifoliate)
Genotype	'Sour' orange (SO)	TH-051
	'Sunki' mandarin (SM)	TH-127
	'Sunki Tropical' mandarin (STM)	'Swingle' citrumelo (SWCT)
	'Rangpur' lime (RL): Aluminum selection 01 (RL Al. 01)	LRF × (RL × TR) - 004
	'Rangpur' lime (RL): Aluminum selection 02 (RL Al. 02)	LRF × (RL × TR) - 005
	'Rangpur Santa Cruz' lime (RLSTC)	SM × TRFD - 007
	RL × SRT-034	SM × SWCT - 041
	SMFL × CWEB-004	SM × (RL × TR) - 016
	SMFL × CTC 13 – 012	SM × CTTR - 002
	SMFL × CTARG-044	SM × CTARG - 020
	VL 71164	SM × CTQT 1434 - 001

*CWEB, Citrus webberi; VL, 'Volkamer' lemon; TH, trifoliate hybrid; TRFD, Trifoliate 'Flying Dragon'; TR, trifoliate; CTARG, 'Argentina' citrange; SMFL, 'Sunki Florida' mandarin; LRF, 'Florida Rough' lemon; CTTR, 'Troyer' citrange; CTC, 'Carrizo' citrumelo; CTQT, 'Thomasville' citrangequat.

et al., 2010). Instruments such as portable scanners and optical laser are designed for measurements of leaf area index (LAI). However, many times, they are very expensive and complex for basic studies (Serdar and Demirsoy, 2006) and involve destructive measures, what makes the sequential readings inviable (Cristofori et al., 2008).

Citrus breeding programs have generated several hybrids, which should be evaluated for tolerance to abiotic and biotic stress and the leaf area is constantly assessed and correlated with most of others physiological traits. Thus, the present study aimed to develop an accurate mathematical model to estimate single blade leaf area, easily applicable and adaptable to any hybrid of *Citrus*.

MATERIALS AND METHODS

Genotypes used and growing conditions

This study was conducted with 22 genotypes of Genetic Breeding Program of citrus (GBP Citrus) of the Embrapa Cassava and Fruits, being classified into two groups according to the leaf types: Simple and compound (Table 1). The leaves of each genotype were collected in five plants of each genotype cultivated in greenhouse during a year, in pots of 40 L.

Modeling and statistics of the results

From each genotype, 22 to 49 leaves were randomly collected sampling the maximum range of scope as possible. The leaf area of each leaf was determined using the methodology of Marshall (1968).

For the simple leaves, the maximum length of the leaf (L) and the maximum width of the leaf (W); for the compound leaves, the maximum lengths of the central folioles (L_1) and lateral (L_2 and L_3) and the maximum widths of the central folioles (W_1) and lateral (W_2 and W_3) were considered. Through the software Table curve, the biometric measurements were treated as independent variables and the leaf area as the dependent variable. The best models were selected based on the coefficient of determination (r^2) (Table 2).

To increase the accuracy of the models for each type of leaf (simple and compound), they were separated into groups according to the form of the leaf blade. In the case of genotypes with single leaves, the criterion used was the relationship between the leaf length by its width (L/W) raised to the second power $(L/W)^2$, obtaining the groups: Group 1: $3 \leq (L/W)^2 \geq 4$; Group 2: $4.1 \leq (L/W)^2 \geq 4.7$, and Group 3: $4.8 \leq (L/W)^2 \geq 6$ (Table 3). For genotypes with compound leaves, was used the ratio between the sum of the length of lateral folioles and the length of the central folioles $(L_2+L_3)/L_1$, with the formation of the following groups: Group 1: $0.8 \leq (L_2+L_3)/L_1 \geq 0.89$; Group 2: $0.9 \leq (L_2+L_3)/L_1 \geq 1$; Group 3: $1.1 \leq (L_2+L_3)/L_1 \geq 1.3$ (Tables 4 and 5).

In order to compare the models proposed, besides the correlations analysis, we calculated the total errors of the estimate of the leaf area and their relative errors. The total error of the estimate for each model generated was calculated by means of Equation (1):

$$E = \sum_1^n Am - \sum_1^n Ar \quad (1)$$

In which E is the total error of estimate of leaf area (cm²); Am is the estimated leaf area (cm²); and Ar is the leaf area measurement (cm²).

The relative error was calculated by the ratio between the difference of the sum of the estimated leaf area ($\sum_1^n Am$) and the corresponding measured value (($\sum_1^n Ar$) by the sum of the real leaf area ($\sum_1^n Ar$) (Equation 2):

$$RE = \left| \frac{\sum_1^n Am - \sum_1^n Ar}{\sum_1^n Ar} \right| \quad (2)$$

In which RE is the relative error (%); ($\sum_1^n Am$) the sum of leaf area, of all the leaves in a genotype, estimated by the proposed model (cm²) and ($\sum_1^n Ar$) the sum of leaf area, considering all the leaves in a genotype (cm²).

RESULTS AND DISCUSSION

Adjusted models

The mathematical models presented the best adjustments

Table 2. Description of models obtained, where β and μ are constants estimated by the software table curve.

Model	Leaves form	
	Simple	Compound
1	$Y = \beta \cdot L \cdot W$	$Y = \beta \cdot (L_1 \cdot W_1)$
2	$Y = \beta \cdot (L \cdot W)^{\mu}$	$Y = \beta \cdot (L_1 \cdot W_1 + L_2 \cdot W_2 + L_3 \cdot W_3)$
3	$Y = \beta \cdot L^{\mu}$	$Y = \beta \cdot [(L_1 + L_2 + L_3) \cdot W_1]$
4		$Y = \beta \cdot (L_1 \cdot L_2 \cdot L_3)^{\mu}$
5		$Y = \beta \cdot (L_1 + L_2 + L_3)^{\mu}$
6		$Y = \beta \cdot {L_1}^{\mu}$

L, Maximum length of the leaf; W, maximum width of the leaf; L_1, maximum length of the central foliole; L_2 and L_3, maximum length of lateral folioles; W_1, maximum width of the central foliole; W_2 and W_3, maximum width of the lateral folioles.

Table 3. Number of leaves (NL) and total leaf area (TLA- cm^2) of genotypes, Constants β and μ of models; coefficients of determination (r^2); ratio of the length and width raised to the second power $(L/W)^2$ and grouping of genotypes (single leaves).

Genotype	NL	TLA	Model 1		Model 2			Model 3			$(L/W)^2$	G
			β	r^2	β	μ	r^2	β	μ	r^2		
STM	45	530	0.709	0.988	0.769	0.974	0.992	0.503	1.819	0.964	3.69	1
SO	26	741	0.687	0.983	0.794	0.964	0.992	0.388	1.970	0.917	3.73	1
RL × LRF -034	29	665	0.693	0.979	0.654	1.016	0.989	0.265	2.133	0.926	3.96	1
SMFL × CWEB-004	37	578	0.692	0.976	0.771	0.968	0.989	0.354	2.049	0.953	3.30	1
SM	41	732	0.713	0.989	0.797	0.969	0.994	0.377	2.020	0.958	3.41	1
VL 71164	27	735	0.723	0.993	0.697	1.010	0.998	0.224	2.231	0.979	3.95	1
RLSTC	43	614	0.675	0.997	0.667	1.000	0.992	0.496	1.756	0.954	4.30	2
SM × CTARG - 044	49	339	0.667	0.986	0.78	0.964	0.967	0.424	1.802	0.952	4.66	2
SMFL × CTC 13-012	40	305	0.670	0.994	0.811	0.935	0.990	0.398	1.913	0.964	4.03	2
RL Al. 02	29	637	0.739	0.994	0.748	0.996	0.997	0.295	0.38	0.988	4.85	3
RL Al. 01	32	696	0.729	0.996	0.758	0.990	0.996	0.429	1.862	0.995	5.50	3
β average			0.700		0.746			0.378				

* ME, Mean absolute and relative error; E, error (if positive; the model overestimated and if negative; the model underestimated the leaf area); R.E., Relative error (represents the percentage of over or underestimate of the model); Model 1: $Y = \beta \cdot (L \cdot W)$; Model 2: $Y = \beta \cdot (L \cdot W)^{\mu}$; Model 3: $Y = \beta \cdot (L)^{\mu}$.

were linear and potential, so they were selected for more detailed analysis. For genotypes with single leaves, three models were chosen: one linear and two potentials; for genotype with compound leaves, six were chosen: three linear and three potential (Table 2).

Models for genotypes with single leaves

All equations of the models individually generated for the genotypes possessing single leaves presented r^2 above 0.9 (Table 3). The constant μ of Model 2 (simple leaf blade) tended to unity, showing that, regardless of the format of the leaf, leaf area is approximately 70% of the area of the rectangle (L.W), with no gains in accuracy with the use of the potential model. When only the length of the midribs as independent variable is used (model 3), the lowest value for constant μ was approximately 1.8, being characterized as potential (Table 3).

As shown in Figure 1, the adjusted models considering the three leaf groups (simple leaf), explained very well the variation of the data presenting excellent adjustment to mathematical models $r^2 \geq 0.99$. It was noticed a proximity to responses of the models when analyzing range in the abscissa axis corresponding to small leaves ($L \cdot W \leq 30$ cm, $L \leq 5$ cm) (Figure 1A to C). Consequently, the procedure of grouping, expressed by the ratio $(L/W)^2$, promotes gains in estimates of LA, especially for larger leaves, range in which there is a greater dispersion of the models, regardless of the genotype tested. When considering the leaves grouping based on the relation $(L/W)^2$, it was possible even the distinction of the access selected from a genotype, as the case of Rangpur lime (RL), in which the selections Aluminum 01 and 02 (group 3) belonged to distinct groups of Santa Cruz (RLSTC) (Group 2) (Table 3).

Table 4. Sum of the errors of the estimation of leaf area (E) of genotypes of single leaves, relative errors (RE) and coefficient of determination (r^2).

Genotype	E (cm²)	Model 1		Model 2			Model 3			G
		RE (%)	r²	E (cm²)	RE (%)	r²	E (cm²)	RE (%)	r²	
STM	-6.19	1.23	0.99	-0.66	0.13	0.99	9.97	1.99	0.95	1
SO	13.26	1.78	0.98	7.91	1.07	0.98	0.38	0.05	0.88	1
RL x LRF -034	11.22	1.69	0.98	9.91	1.49	0.98	32.91	4.94	0.88	1
SMFL x CWEB-004	7.09	1.23	0.98	9.98	1.73	0.98	-22.93	3.97	0.94	1
SM	-11.81	1.61	0.99	-9.50	1.30	0.99	-38.72	5.29	0.92	1
VL 71164	-20.86	2.84	0.99	-26.46	3.60	0.99	-22.07	3.00	0.97	1
RLSTC	15.34	4.78	0.99	17.92	5.59	0.99	60.36	18.83	0.98	2
SM x CTARG – 044	-2.63	0.78	0.99	20.01	5.91	0.99	70.20	20.73	0.95	2
SMFL x CTC 13-012	16.21	5.31	0.99	19.22	6.31	0.99	21.65	7.09	0.95	2
RL Al. 02	-3.95	0.62	0.99	-31.24	4.90	0.99	88.31	13.81	0.98	3
RL Al. 01	4.81	0.69	0.99	-24.77	3.56	0.99	106.80	15.27	0.97	3
*ME.	10.12	1.23		16.21	1.90		39.84	6.20		

The estimates were based on the models proposed for each group presented in Figure 1.* ME, Mean absolute and relative error; E, Error (if positive, the model overestimated and if negative, the model underestimated the leaf area); R.E., Relative error (represents the percentage of over or underestimate of the model); Model 1: $Y = \beta \cdot (L \cdot W)$, Model 2: $Y = \beta \cdot (L \cdot W)^{\mu}$, Model 3: $Y = \beta \cdot (L)^{\mu}$.

Table 5. Number of leaves (NL), total leaf area (TLA -cm²).

Genotype	NL	TLA	Model 1		Model2		Model 3		L: $(L_2+L_3)/L_1$	G
			β	r²	β	r²	β	r²		
SM x (RL x TR) - 016	31	495	1.051	0.86	0.731	0.84	0.560	0.93	0.857	1
SM x CTTR - 002	38	492	1.046	0.86	0.779	0.96	0.559	0.96	0.808	1
TH - 051	26	330	1.170	0.97	0.790	0.99	0.617	0.99	0.985	2
SWCT	22	431	1.229	0.94	0.768	0.99	0.590	0.99	1.120	3
TH - 127	22	340	1.263	0.90	0.777	0.98	0.602	0.96	1.100	3
SM x SWCT - 041	28	466	1.273	0.85	0.802	0.97	0.598	0.95	1.132	3
LRF x (RL x TR) - 004	29	432	1.266	0.90	0.773	0.94	0.599	0.93	0.159	3
SM x TRFD – 007	30	349	1.230	0.92	0.752	0.98	0.579	0.96	1.121	3
LFR x (RL x TR) - 005	31	409	1.287	0.89	0.784	0.97	0.596	0.93	1.187	3
SM x CTQT 1431 - 001	38	294	1.254	0.87	0.788	0.97	0.587	0.95	1.197	3
SM x CTARG - 020	35	384	1.205	0.93	0.745	0.97	0.562	0.95	1.130	3
β average			1.207		0.771		0.586			

Constant β used on linear models and respective coefficients of determination; average of the sum of the lateral lengths divided by the central and grouping of genotypes (compound leaves). Model 1: $Y = \beta \cdot (L_1 \cdot W_1)$, Model 2: $Y = \beta \cdot (L_1 \cdot W_1 + L_2 \cdot W_2 + L_3 \cdot W_3)$, Model 3: $Y = \beta \cdot [(L_1 + L_2 + L_3) \cdot W_1]$.

The estimate errors for each genotype, from the use of the adjusted models for each group (Figure 1), are presented in Table 4. In the case of the linear model (Y = β. (L.W)) they were lower in relation to the two powers (Models 2 and 3), with ER ranging from 0.62% to aluminum RL 02 to 5.31% for SMFL x CTC 13-012; and the average deviation the lowest among the three models tested (Table 4). The third model, in which it was used only the length (L) as the independent variable, proved to be comparatively less precise, especially for the genotypes belonging to groups 2 and 3. That result indicates the need of use of all the variables L and W in the estimates of single leaves for a greater precision regardless of the group. Considering that there were different responses depending on the genotype evaluated, with relative error (RE) minimum of 0.05% for SO and maximum of 20.73% for SM x CTARG - 044, proportionally different from the models which used L.W as input variable □ 6.31%. Among the models for simple leaf format, the most appropriate was the linear (Y = β. (L.W)). The advantages are by the high precision on the estimates and ease of practical application, confirming

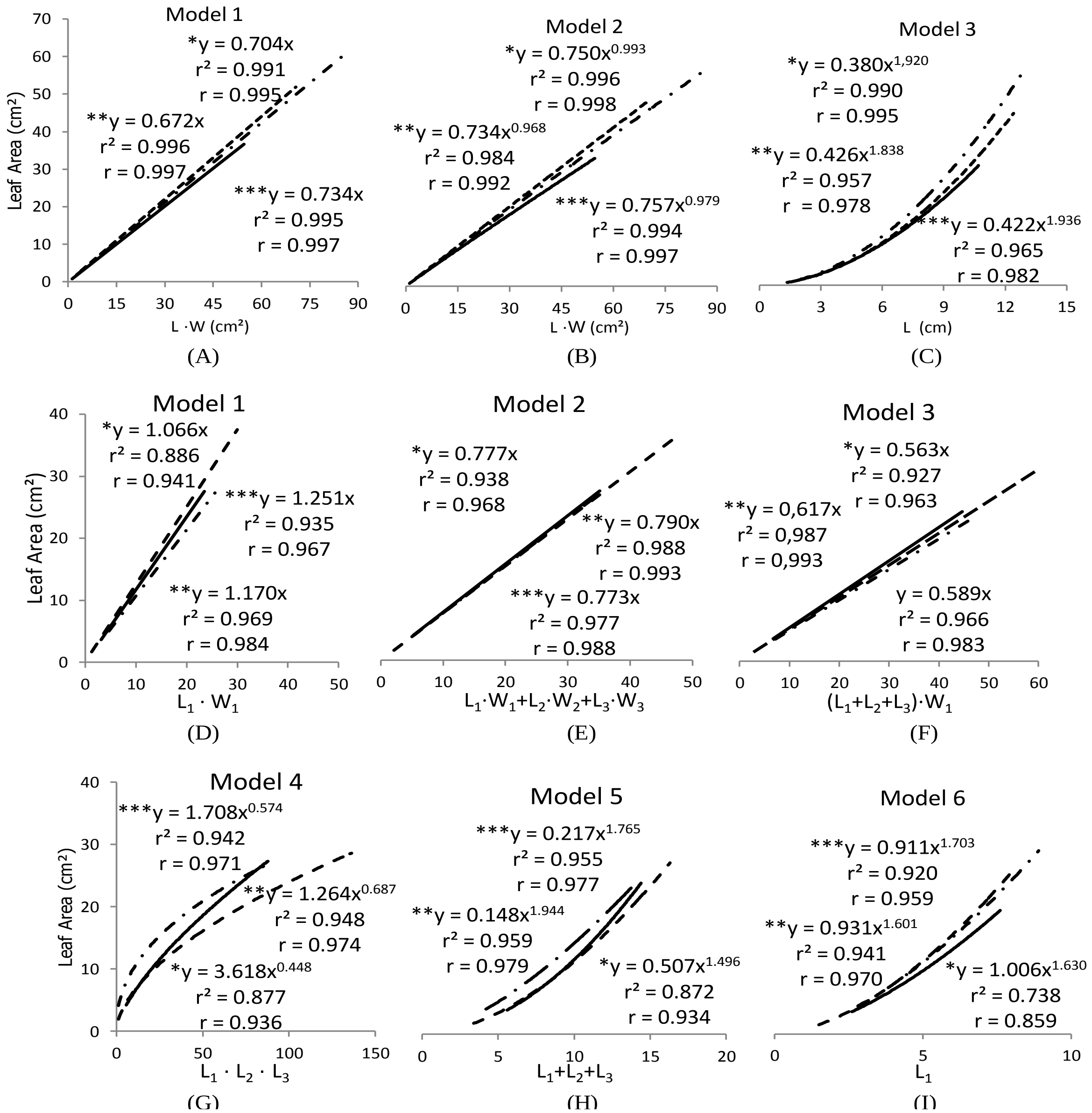

Figure 1. Regressions fitted for grouped genotypes (A to C - single leaves and D to I - compound leaves) and their coefficients of determination (-··Group 1* - Group 2**, - - -Group 3***, L- maximum length of the leaf, W - maximum width of the leaf, L_1 - maximum length of the central foliole, L_2 and L_3 - maximum length of lateral folioles, W_1 - maximum width of the central foliole, W_2 and W_3 - maximum width of the lateral folioles; axis Y of same scale for the graphics from A to C and D to I).

and justifying its widespread use in the estimate of leaf area in different plant species (Blanco and Folegatti, 2005; Coelho Filho et al., 2005, 2012, Cristofori et al., 2007; Malagi et al., 2010; Sousa et al., 2014; Souza and Amaral, 2015).

Models for compound leaves genotypes

The mathematical models tested fitted well for all genotypes, by the values of $r^2 \geq 0.84$ (Tables 4 and 5). The choice of mathematics ratio $(L_2+L_3)/L_1$, originally based on visual observations of variability, was attested by the high correlation with the constant β, model 1 (Table 5), Spearman's correlation coefficient of 0.98 (figure not shown).

Considering only the linear models (Table 5), there was less variation in the amplitude of the values of the constant β in the third model proposed; therefore, the model was sensitive to changes in the shape of leaves. In a converse way, variations were greater for the first model. Such results probably reflect the number of variables used in each model.

Analyzing the estimates of leaf area within each

Table 6. Number of leaves (NL), total leaf area (TLA-cm²).

Genotype	NL	TLA	Model 4			Model 5			Model 6			G
			β	μ	r^2	β	μ	r^2	β	μ	r^2	
SM × (RL × TR) - 016	31	495	3.458	0.48	0.94	0.321	1.73	0.93	0.764	1.84	0.86	1
SM × CTTR - 002	38	492	3.510	0.42	0.91	0.543	1.42	0.93	0.991	1.58	0.86	1
TH - 051	26	330	1.264	0.69	0.95	0.148	1.94	0.96	0.931	1.60	0.94	2
SWCT	22	431	1.705	0.61	0.92	0.169	1.92	0.94	0.703	1.92	0.87	3
TH - 127	22	340	1.418	0.62	0.92	0.114	2.01	0.95	0.535	2.01	0.84	3
SM × SWCT - 041	28	466	2.368	0.50	0.94	0.337	1.60	0.95	0.866	1.75	0.88	3
LRF × (RL × TR) - 004	29	432	1.981	0.54	0.92	0.291	1.65	0.94	1.260	1.51	0.94	3
SM × TRFD - 007	30	349	1.958	0.52	0.93	0.265	1.66	0.95	0.852	1.71	0.89	3
LFR × (RL × TR) - 005	31	409	1.834	0.55	0.96	0.281	1.65	0.97	1.192	1.54	0.95	3
SM × CTQT 1431 - 001	38	294	1.827	0.51	0.94	0.294	1.58	0.96	1.072	1.53	0.94	3
SM × CTARG - 020	35	384	1.842	0.54	0.97	0.251	1.69	0.97	0.969	1.63	0.88	3
β and μ averages			2.106	0.55		0.274	1.71		0.921	1.70		

Constant β and μ used on models and their respective coefficients of determination, and grouping of genotypes (compound leaves). Model 4: $Y = \beta \cdot (L_1 \cdot L_2 \cdot L_3)^{\mu}$, Model 5: $Y = \beta \cdot (L_1 + L_2 + L_3)^{\mu}$, Model 6: $Y = \beta \cdot (L_1)^{\mu}$

Table 7. Sum of errors, relative errors, coefficient of correlation between the area of each leaf and the estimated area to the linear models of genotypes of compound leaves estimates based on the specific model for each group presented in Figure 1.

Genotype	Model 1			Model 2			Model 3		
	E (cm²)	RE (%)	r²	E (cm²)	RE (%)	r²	E (cm²)	RE (%)	r²
SM × RL × TR) - 016	0.00	0.00	0.93	19.99	4.04	0.93	-4.55	0.92	0.97
SM × CTTR - 002	3.78	0.77	0.93	-6.40	1.30	0.98	2.95	0.60	0.98
TH - 051	-5.92	1.79	0.99	-3.07	0.93	1.00	-2.52	0.76	0.99
SWCT	2.42	0.56	0.97	-0.27	0.06	0.99	0.61	0.14	0.99
TH - 127	-10.19	3.00	0.96	-4.22	1.24	0.99	-11.56	3.40	0.99
SM × SWCT - 041	-10.04	2.15	0.93	-15.62	3.35	0.98	-5.97	1.28	0.97
LRF × (RL × TR) - 004	0.57	0.13	0.97	17.30	4.00	0.98	4.13	0.96	0.98
SM × TRFD - 007	-0.32	0.09	0.97	5.71	1.64	0.99	-0.36	0.10	0.98
LFR × (RL × TR) - 005	-25.03	6.11	0.96	-13.93	3.40	0.99	-16.79	4.10	0.97
SM × CTQT 1431 - 001	-12.71	4.32	0.96	-10.89	3.70	0.99	-5.84	1.98	0.99
SM × CTARG - 020	5.88	1.53	0.97	7.28	1.90	0.99	7.60	1.98	0.98
*ME	7.35	1.48		9.43	1.25		4.97	1.01	

*ME, Mean absolute and relative error, E, Error (if positive, the model overestimated and if negative, the model underestimated the leaf area); R.E., Relative Error (represents the percentage of over or underestimate of the model); Model 1: $Y = \beta \cdot (L_1 \cdot W_1)$, Model 2: $Y = \beta \cdot (L_1 \cdot W_1 + L_2 \cdot W_2 + L_3 \cdot W_3)$, Model 3: $Y = \beta \cdot [(L_1 + L_2 + L_3) \cdot W_1]$.

genotype, based on the adjusted models from each group (Figure 1), it was observed that the largest number of independent variables used in Model 2 reflected the higher values for the coefficient of determination, except in SM x (RL x TR) genotype - 016, in which it was noticed the best fit when using the third model (Table 5). Possibly the greatest number of independent variables of the model 2 increased its sensitivity, regardless of the leaf groups (1, 2 and 3), expressed by the proximity of the angular coefficients obtained (Figure 1E). That result suggested the feasibility of using an average value, regardless of grouping.

Considering that observation, a single regression with the data of 11 genotypes of compound leaves based on that model $(Y = \beta \cdot (L_1 \cdot W_1 + L_2 \cdot W_2 + L_3 \cdot W_3)$ was performed. The value of β is equal to 0.776 and the model explained very well to the values observed by the coefficient of determination of 0.976 (figure not shown). In that case, in function of response independent of the genotype, the lack of concerning with groupings is a positive point. However, there is a need for a greater number of independent variables, which can restrict its use in practice, when the goal is to perform a large number of measures.

The proximity of the results with the use of linear models (Table 7) (average deviation of the relative error

Table 8. Sum of errors, relative errors, coefficient of determination between the average area of each leaf and the estimated area for the potential models of genotypes of compound leaves.

Genotype	Model 4			Model 5			Model 6		
	E (cm²)	RE (%)	r²	E (cm²)	RE (%)	r²	E (cm²)	RE (%)	r²
SM x (RL x TR) - 016	56.29	11.38	0.92	56.07	11.33	0.93	-6.70	1.35	0.84
SM x CTTR – 002	-35.95	7.30	0.97	-44.59	9.06	0.97	-96.44	19.59	0.78
TH – 051	-1.44	0.44	0.97	-1.45	0.44	0.97	-2.30	0.70	0.96
SWCT	-63.63	14.78	0.94	-58.40	13.56	0.95	-56.96	13.23	0.91
TH – 127	-5.46	1.61	0.95	-2.78	0.82	0.97	-0.05	0.01	0.91
SM x SWCT – 041	-13.07	2.80	0.93	-11.43	2.45	0.94	-18.84	4.04	0.89
LRF x (RL x TR) – 004	12.82	2.97	0.95	15.78	3.65	0.96	8.66	2.00	0.95
SM x TRFD – 007	14.18	4.07	0.95	15.05	4.32	0.96	16.44	4.71	0.94
LFR x (RL x TR) – 005	7.59	1.86	0.96	4.98	1.22	0.96	-9.47	2.31	0.96
SM x CTQT 1431 – 001	31.96	10.86	0.94	26.17	8.89	0.96	17.44	5.93	0.96
SM x CTARG – 020	8.91	2.32	0.97	8.53	2.22	0.97	10.04	2.62	0.94
*ME	22.74	4.07		22.23	3.96		24.46	4.24	

Estimates based on model specific to each group presented in Figure 1. *ME, Mean absolute and relative error; E, Error (if positive, the model overestimated and if negative, the model underestimated the leaf area), R.E., Relative error (represents the percentage of over or underestimate of the model); Model 4: $Y = \beta \cdot (L_1 \cdot L_2 \cdot L_3)^{\mu}$, Model 5: $Y = \beta \cdot (L_1 + L_2 + L_3)^{\mu}$, Model 6: $Y = \beta \cdot (L_1)^{\mu}$.

(RE) ranging from 1.01 to 1.48; and average deviation of error (E) ranging from 4.97 to 9.43), justifies the use of the Model 1 $Y = \beta \cdot (L_1 \cdot W_1)$, due to its greater simplicity and practicality, confirming one more time the widespread use by different authors.

Analyzing the potential models, it was found that the constant μ for groups in the leaf model 4 ($Y = \beta \cdot (L_1 \cdot L_2 \cdot L_3)^{\mu}$ were lower than one (Table 6), suggesting a reduction in the estimate rate of leaf area according to the increase of leaf length (Figure 1 G), what can cause major errors in the estimate of the area of leaves with high length. On fifth and sixth models, once the exponents are larger than the unit (Table 6), the angular coefficient of the tangent lines to the curve increases with the elevation of the value of the input variable, the opposite of what happened in the fourth model (Figure 1G to I).

Despite the high accuracy of the estimates obtained individually for the genotypes, in relation to the potential models 4, 5 and 6, according to the coefficients of determination (Table 8), when analyzing the statistical parameters 'average error' and 'standard error', there is a greater precision and accuracy when used with the linear models (Tables 7 and 8).

In a general way, the estimates of leaf area for all genotypes using the six models proposed resulted in high coefficients of determination (>0.88). Exception for SM x CTTR - 002 and SM x (RL x TR) - 016, with respective values of 0.78 and 0.84; both for the sixth model. When compared only the potential models, the fifth model presented the best adjustment, lower errors of estimate (E and ER) and a higher r² (Table 8). Among the linear models, due to the proximity of the errors and high values of r^2, the model $Y = \beta. L.W$ is very interesting for the greater ease of practical determination, favoring the largest sample in studies of plant growth. Earnings comparatively small in accuracy can be obtained with the use of the linear models 2 and 3 ($Y = \beta \cdot (L_1 \cdot W_1 + L_2 \cdot W_2 + L_3 \cdot W_3)$, Model 3: $Y = \beta \cdot [(L_1 + L_2 + L_3) \cdot W_1]$, despite the larger number of variables to be measured. Compared to the linear, it can provide errors of estimates higher than the linear, for some genotypes. Different types of mathematical models have been generated for different plant species and leaf type. However, the models are developed for specific species and are usually restricted to few varieties or form of leaf, as performed by Coelho Filho et al. (2005, 2012); Malagi et al. (2010); Souza and Amaral (2015) and Toebe et al. (2012). In the present study different mathematical models of leaf area estimate with different levels of accuracy were developed, with advantages of being applicable to any genotype of citrus just requiring the knowledge of biometric relations that differentiate the leaf shape.

Conclusions

The greater precision of estimates is achieved when using specific models for each type (simple and compound leaves) and separating these types in homogeneous groups in relation to leaf dimensions and folioles. For simple and compound leaves, the respective linear models ($Y = \beta. L. W$; $Y = \beta.L_1.W$) showed the best statistical performance, besides being easy to use. The potential models $Y= \beta L^{\mu}$ and $Y= \beta L1^{\mu}$, respectively for simple and compound leaves, require only one input biometric variable, which in a practical way, allow an increase in the number of repetitions, but provide errors of estimates higher than linear models. The model $Y = \beta (L_1 \cdot W_1 + L_2 \cdot W_2 + L_3 \cdot W_3)$ has been sensitive in the estimates of leaf area, independent of the grouping of compound leaves genotypes, for a $\beta = 0.7755$.

Conflict of interests

The authors have not declared any conflict of interests

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